PHYSIOLOGICAL AND MICROBIAL STUDIES IN A WEED, *ARISTOLOCHIA BRACTEOLATA* LAM.

Thesis Submitted

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FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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DECLARATION

I hereby declare that the work incorporated in the thesis entitled "Physiological and microbial studies in a weed, *Aristolochia bracteolata* Lam." has not been submitted in part or full by me for degree or diploma of any degree or diploma of any other university or institute.

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ABBREVIATIONS

2, 4- D	2, 4 Dichlorophenoxy acetic acid
DCPIP	2,6 Dichlorophenol Indophenol
ABA	Abscicic Acid
Abs	Absorbance
ADP	Adenosine diphosphate
ATP	Adenosine Triphospahate
ATPase	Adenosine triphosphatase
ATP	Adenosine Triphosphate
AEC	Anion Exchange Capacity
AM	Arbiscular Mycorihiza
AA	Ascorbic acid
AAS	Atomic Absorption Spetrophotometer
amu	atomic mass unit
BC	Before Christ
β	Beta
BSC	Biological Soil Crust
BSA	Bovine Serum Albumin
Ca	Calcium
CaSI	Carotenoid Stability Index
CAT	Catalase
CEC	Cation Exchange Capacity
cm	Centimetre(s)
ΔOD	change in optical density
CSI	Chlorophyll Stability Index
Chl	Chlorophyll(s)
n	Chromosome number
et al.	Co- workers
Co -A	Coenzyme- A

CFU	Colony Forming Unit
Conc.	concentrated
Cu	Copper
CAM	Crassulacean acid metabolism
CAM	Crassulacean Acid Metabolism
cv	Cultivar (s)
DSF	Dark Septate Fungi
dS m-1	Deci semen per meter
0C	Degree Celsius
DNA	Deoxyribose nucleic acid
d^6	Deteriorated
Dia.	Diametre
DTPA	Diethylenetriaminepentaacetic acid
d.f.	Dilution factor
DMSO	Dimethyl Sulphoxide
DI	Direct Injection
D. W.	Distilled Water
DW	Distilled Water
Dry.Wt.	Dry Weight
E	East
EC	Electrical conductivity
ETS	Electron Transport System
eV	Electron volt
EI	Electronic impact Ionisation
EDTA	Ethylene diamine tetra acetic acid
Fig .	Figure(s)
FAD	Flavin Adenine Dinucleotide
FT-IR	Fourier transform infrared spectroscopy
F. W.	Fresh weight
GCMS	Gas Chromatography and Mass Spectroscopy
GC-MS	Gas Chromatography- Mass Spectroscopy

GDH	Glutamate Dehydrogenase
GS	Glutamine Synthetase
g	Gram(s)
>	Greater than
HPLC	High performance liquid chromatography
H	Hour(s)
pH	Hydrogen ion concentration
H2O2	Hydrogen peroxide
IAAO	Indole Acetic Oxidase Oxidase
IR	Infra Red
Fe	Iron
JCPDS	Joint Committee on Powder Diffraction
kDa	kilo Dalton(s)
kg	kilogram(s)
Kg/ ha	Kilograms per hectare
Km	Kilometer
<	Less than
LC	Liquid Chromatography
Mg	Magnesium
Mn	Manganese
m/z	Mass by charge ratio
MHz	Megahertz
Sm	Mesophyll Succulence Index
mRNA	messenger RNA
m	metre (s)
µg	Microgram(s)
µg	micrograms
µl	microlitre
µm	Micrometre (s)
µM	Micromola
µ	Micron(s)

meq	miliequivalents
ml	Mililitre (s)
mm/h	milimetre per hour
mM	Milimolar(s)
mg	milligram(s)
mm	Millimetre (s)
min	Minute(s)
M	Molar
mol	Mole(s)
M.W.	molecular weight
NEEDA	N(1-Napthyl) ethylene diamide hydrochloride
nm	Nanometre (s)
nMole	nanomoles
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (Reduced)
NADH	Nicotinamide adenine dinucleotide reduced
NR	Nitrate Reductase
NiR	Nitrite Reductase
N	Normal
N	North
NTP	Nucleotide triphosphate
No.	Number
OD	Optical Density
OC	Organic Carbon
OM	Organic Matter
O.P.	Osmotic Potential
PCA	Paracoumaric acid
ppm	Parts per million
g-1	Per gram
m-2	Per metre square
%	Percentage

POX	peroxidase
PS-I	Photosystem I
C3	Plant operating Calvin path of photosynthesis
C4	Plant operating Hatch, Slack and Kortschak path of photosynthesis.
PEG	Polyethylene Glycol
PPO	Polyphenol oxidase
K	Potassium
PDA	Potato Dextrose Agar
^{1}H-NMR	Proton Nuclear Magnetic Resonance spectroscopy
RBD	Randomized Block Design
R.M.	Reaction Mixture
ROS	Reactive oxygen species
RWC	Relative Water Content
Resp.	Respectively
rpm	Revolutions per minute
Sec.	Seconds
Na	Sodium
SAR	Sodium Absorption Ratio
SOM	Soil Organic Matter
sp.,	spp Species
SI	Succulence Index
S	Sulphur
SOD	Superoxide dismutase
TLC	Thin layer chromatography
TL	Thraumatin Like Protein
TAN	Titratable Acidity Number
TOC	Total Organic Carbon
TCA	Trichloro acetic acid
TTC	Triphenyl Tetrazolium Chloride
UV	Ultraviolet

UK	United Kingdom
USA	United States of America
var	Variety
viz.	*Videlicet* = namely
v/v	Volume /volume
WUE	Water Use Efficiency
wt.	Weight
w/v	Weight / volume
XRD	X-ray crystallography
Zn	Zinc

CHAPTER I

INTRODUCTION

Plants and their communities are man's most valuable assets, without which, he could not have survived on earth. Out of 3,00,000 species of plants known in the world, hardly 3000 are of economic value to us. When one grows any of the economic species of plants, invariably a variety of volunteer vegetation comes up simultaneously which is competitive and undesirable. Several plants around us are unwanted because either these are responsible for human and animal health hazards or they may influence the vegetation adversely. Immediately one considers such plants as weeds. In this context it is well said that, while all weeds are unwanted plants, all unwanted plants may not be weeds (Gupta, 2005). Of the world's 250000 known flowering plant species, only about 200 are recognized as important weeds (Holm *et al.,* 1997), yet they have a vast impact on a global scale. According to Bell (1905), weed is a plant of outside place. Many weeds are aggressive, troublesome and undesirable elements of the world's vegetation. Shaw (1956) stated that weeds grow in places, where they are not desired. However, no plant is useless in nature. In fact, weeds are important from the standpoint of medicinal, allelopathic and food values (Oudhia and Tripathy, 1999). Thus, it is timely to select several model weed species that will provide the weed science community a focused approach to determine the nature and mechanisms involved in weedy characteristics. Baker (1974) proposed the idea of ''general-purpose genotypes'' in which weeds evolved to encompass multiple weedy characteristics that impart tolerance to different environments and thus have the potential for range expansion. Thus, for a given characteristic different combinations of genes and alleles account for the diversity in phenotypic adaptation required for survival. Logically, general-purpose genotypes must result from the quantitative genetic control of characteristics that account for the tolerance and plasticity of weeds.

Baker (1965) defines a weed as 'a plant if, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without, of course, being a deliberately cultivated plant)'. Weeds are those plants that are successful in disturbed environments, fast growing, and often but not always herbaceous (Zimdahl, 1992). A weed can be defined as 'a plant or a group of plants which is not desired at its place of occurrence' (Pieterse and Murphy, 1990). Weeds are adapted to habitats disturbed by man. They may be useful in some respects and harmful in others. They may be useful to some people and hated and despised by others. There are weed races of most of our field crops and these interact genetically

with cultivated as well as truely wild races. This interaction probably results ultimately in better crops and more persistent weeds. Although some weeds have evolved elegant adaptations under the influence of human activity, many wild species had weedy tendencies before man existed. Weeds are products of organic evolution. They are also genetically labile and phenotypically plastic. Weeds have been constant and intimate companions of man throughout his history and could tell us a lot more about man, where he has been and what he has done, if only we knew more about them (Harlan, 1992).

Defining what a weed is, and appreciating the traits they possess, is a good start to understand the nature of these plants. Evolution acts on individuals in a population, so as to realise the nature of the weeds we have today, it requires a thorough understanding of where and when particular plant species became weed.

Most of the common and widespread weed species we know have came as a consequence of crop domestication, planting and cultivation. These agricultural processes began about 12,000 years ago. Agricultural development occurred on different continents independently and involved different native species available for selection as crops. Since, those early originated, both crops and their weeds have spread throughout the world. These crop-weed groups are the most successful invasive species in human history. Recent studies in some countries showed that weed species may have other economic importance as is also often disregarded or unknown to policy makers. The importance of plant's use as medicine should be taken into account when a plant is considered for listing as a declared weed or declared invader under the conservation of Agricultural Resources Act (Dold & Cocks, 2000). Ethenobotanical studies reveal how human beings have utilized plants for primary survival and aesthetic purposes. It is the science that covers historical as well as present usage of plants (Nyazema, 1996). Ethenbotanical studies are often significant in revealing locally important plant species especially for the discovery of crude drugs (Cox & Balick, 1996; Flaster, 1996). Weed species form a component of agro-biodiversity, playing a part in the ecology of natural enemies as in harbouring and supporting many beneficial arthropod species that suppress pest populations consequently improving crop yields. Such weeds offer alternative prey/hosts, pollen or nectar and microhabitats for natural enemies (Altieri, 1994). Crop fields with dense weed cover and high diversity usually have more predacious arthropods than do weed-free fields (Stephen 1982; Espanol, 1976; Altieri, 1994).

Weed science, especially in Eastern Africa and other parts of tropical regions, has tended to concentrate on ecology, taxonomy and control principles of general weed (Wild 1968; Terry *et al.,* 1984; Terry & Michieka, 1987; Baker & Terry, 1991; Jiro, *et al.,* 1997). Management agencies in Eastern Africa have began to recognize the importance of invasive species and weeds, and mentioned many of them as good sources of medicines. Obviously, this has prompted the current study so as to point out what weed plants need is careful consideration for conservation rather than eradication. The data is also useful in providing species that needs to be re-evaluated for cultivation under controlled conditions. Hence, by increasing agro biodiversity and accessible medicinal plant products, India, as a subcontinent harbours greater number of weeds as compared to any other adjacent country. This is primarily due to vast array of ecological habitats having climatic, edaphic and topographical factors present in India. Weeds are unwanted plants growing in places where they are not required, but majority of them are useful medicinally and economically. Several weeds spread like wildfire, grow abundantly, gregariously in the open spaces, roadsides, crop fields etc. and also grow in all type weather conditions. But rainy and winter seasons are most productive for their growth and spread. By careful screening and analysis, these weeds would be of great source of herbal medicines.

The World Health Organization has estimated that 80% of the world's population relies on traditional healing modalities and herbs. Many of these traditional medicinal herbs usually grow as weeds. The role of weeds in the present pharmacopeia has been overlooked, despite significant evidence that weeds in particular, are an important source of medicines for indigenous people and have a highly significant over representation in indigenous pharmacopoeias in relation to other types of plants (Stepp and Moerman, 2001). A species was considered to be a weed if it was included in the standard reference for weeds worldwide, containing over 8000 species based on a global literature search (Holm *et al.,* 1979). Because it is a global compendium based on a comprehensive survey of the literature, do not offer a singular definition of weeds. However, the authors have classified each species as to whether it is a serious, principal, or common weed; whether its rank is unknown; and whether a species is present in the flora but unconfirmed that it behaves as a weed. For the purpose of this analysis, weeds that were considered to be serious, principal,

or common have been included. Thus weeds are those plants that are successful in disturbed environments, short-lived, fast-growing and oftentimes, herbaceous.

Based on a World Health Organization survey (Farnsworth *et al.,* 1985) and as well as recent review articles for drugs of natural plant product origin (Farnsworth and Soejarto, 1991; Cragg *et al.,* 1997), there are around 121 pharmaceutical compounds used as medicine worldwide that are derived from plants. Of these, 119 compounds from 101 plant species are the primary sources. Using a conservative estimate of 250,000 flowering plants (Heywood, 1993) and an estimate that 8000 of these plants are weeds, one would estimate that 3% of these 101 species would be weeds. However, the actual number is much more than an order of magnitude. Forty-five of the 101 are noted as weedy species (Holm *et al.,*1979*),* the definitive global reference guide for weeds. It should be noted that the overall concept of a "weed" contains a variety of features, not all present in any one weed. However, if we take the definition stated as a yard stick above the vast majority of these species fit this definition, there is increasing evidence to support the hypothesis that weeds are relatively high in bioactive secondary compounds and are thus likely to hold promise for drug discovery. Also, in weeds secondary compounds are important for a variety of ecological novel functions.

Putnam (1994) has reported that at least 50 species of weeds have been shown to interfere with crops through allopathic secondary compounds. However, because allelopathy usually occurs through the complex chemical matrix of the soil, it is difficult to conclusively show a causal relationship (Zimdahl, 1999). Plants with long-lived leaves rely on immobile defences while plants with short-lived leaves invest in toxic compounds. Interestingly, leaves of weeds are generally short-lived, fast-growing species tend to invest in mobile defence compounds rather than immobile (Coley *et al.,* 1985). Obviously Weeds are generally fast-growing. Taken as a whole, it is reasonable to infer from the present data that weedy plant species will usually rely strongly on bioactive defence compounds against herbivores. Weeds have been a problem to man ever since he began cultivating crops about 10,000 BC (Hay, 1974). Sankaran and Mani (1972), reported that in the first 35 days after sowing *Sorghum*, weeds removed almost 46.1, 18.3, and 47.7 kg of N, P and K per ha from the soil respectively, while the crop could take up only 23.8, 9.4 and 46.8 kg / ha of N, P and K. It is well established that weeds are successful competitors for agriculture as both are dependent on the soil on which they are growing, nutrient and water uptake as

these become one of the most vital issue for competition. But the success of weed's survival is in it's capacity to grow even in poor soils. In order to confirm, one has to investigate the aspect of mineral nutrition in these dominant weeds. By forcing agricultural land in natural areas we have created biological imbalance, and so now, in crop land natural plants compete with the beneficial and desired vegetation reducing the yield and quality of the products. These neutralised plants called weeds have become an important factor in the management of all land and water resources; these compete with crop plants for nutrients, soil moisture, space and sunlight. Out of annual loss of agricultural products from pests in India, weed account for 45%, insects 30%, disease 20% and other pests 5% (Rao, 1983). Thus, better agricultural management can become successful only when weed control is effective.

Weeds are the biggest competitor for economic crops, lawn, grass and open places left for beautification. They cause tremendous losses to production. The struggle for existence between weeds and man has been going on from time immortal. It must be kept in mind that nature favours weed species which have large ecological amplitude. Such species multiply and flourish even in adverse environmental conditions such as edaphic factors, microclimate and change the succession of weed species as well. The ecological amplitude of a plant species may get reflected in its morphological, anatomical and physiological behaviour which is flexible over time. We can describe weeds in short as a class representing most successful plant forms, which are aggressive and have been evolved simultaneously with the destruction of habitat and indigenous vegetation by man.

Eventhough, several crops like cereals, pulses and oil crops have evolved from weeds in past (Vavilov, 1992) not all weeds are desirable and many create problems in agricultural process and also in ultimate yield. In order to control their invasion, one has to remove these weeds and for this, we have to see the secret of their success in survivorship. This can only be done by their detailed study of survival potential. Such study may include morphological and anatomical analysis, and it's behaviour, through it's response towards the stresses in the surrounding. This is possible only when we know its physiology and biochemistry. Investigation of weed survival has become vital issue for it's control.

Weeds have an excellent adaptation capability to the disturbed environment in which they are growing. Weeds in contrast to crops generally produce large number

of seeds per plant; they may also possess highly effective method of vegetative reproduction. This gives an idea about their high ability of reproduction, which is also an important dimension of weeds. Enlightening with economic potential of weeds, some authors tend to define weeds as plants for which economic uses are yet to be discovered. Weeds compete with crops for physical resources of environment, exhibit allelopathy, provide habitats for other harmful organisms cause problems during harvest, ploughing and seed purification. Farmers, therefore, control the weeds to enhance productivity. Thus, for effective control, biology of weeds including identification and distribution also deserves attention. The requisite ecological information has always been pre-requisite for all such efforts.

Weed communities, the population that live in them and how they change with time, is one of the most important areas of weed biology. Weed communities are the consequence of particular phenotypes seizing and exploiting opportunity. Much remains to be discovered about how weed populations assemble in communities with crops and how they change over time.

Currently, Howarth (2010) in his work on genomics of weed science states that origin of weed populations at given time cannot be answered through conventional studies and techniques. There is a need of physiological and biochemical studies as this may begin to illuminate the mechanisms and biological processes that make weeds so difficult to control. Plants are underway in several laboratories for microarray analysis to compare transcriptomes of various invasive weeds in their native and introduced ranges, and under common garden conditions to identify gene expression differences related to invasiveness. Physiological processes required for proliferation of weeds and invasiveness should be understood for the establishment of new methods to help weed control, instead of eradication and be further used for its medicinal importance.

Plants that have acclimatised to a certain habitat always show morphological, reproductive and physiological adaptations for successful survival. Morphological adaptations lead to formation of new ecotype and they do not show much flexibility. But they exhibit a dynamic physiological as well as biochemical response towards their surrounding environment (Sen,1981). These physiological responses are mainly in tune with water relations, nutrient uptake and its carbon, nitrogen, developmental

and survival budget. Water relations may show fluctuations due to several physical factors and so they ultimately influence metabolic activities within the plants. This ultimately leads to the formation of strategy by plant and if it can do so becomes successful and dominant survivor.

Photosynthesis is the single most important process involved in productive assimilation of organic carbon for any ecosystem and that in plants photosynthetic CO_2 fixation occurs exclusively through the Calvin cycle. According to Laetsch (1974) CAM plants seem to have adapted to constantly arid environment and represent well in the flora of saline and brackish habitats. Plants have obviously evolved and adapted in response to selective pressure prevalent in xeric and saline habitats. Thus, it seems that CAM mode is a successful strategy for plants growing in drought condition.

Baramati is a drought prone semi-arid region as it is severally affected by rain shadow effect of Western Ghats. Weed flora of any given region always exhibits the progressive domination and through interspecific competition they try to compete among themselves. Furthermore, it also reflects the surrounding microclimate and nature of the habitat. Murumkar and Magdum, (1991), in their project, Ecophysiological studies in some weeds of Baramati region observed that several characteristic weeds grow well and flourish in this region and to surprise, some of these dominate this area even in unfavourable conditions like hot summer. They concluded that in order to understand successful survival of the weed in a given habitat, one must thoroughly investigate its surrounding and possible strategy adapted by the plant and for this one has to look into the various ecophysiological aspects of weed species involved.

Baramati (18^0, 8' N and 75^0, 7' E) is a taluka place of Pune district in Maharashtra, India. This region is a distinct semi-arid zone because of the rain shadow effect of Western Ghats. This place has mean annual maximum temperature 33.47^0C and minimum 20.03 ± 2.00^0C (Murumkar, 1993). Highest daily temperature is recorded during May (mean max.36.95^0C) and lowest in December (mean max.17.07^0C). During summer, especially in April and May, daily maximum temperature many a times shoots up beyond 40^0C. On an average this region is dry with almost 7 months of dry hot period.

The relative humidity is highest during months of September (83.69%) while the lowest recorded in April (55.11%).The rate of evapotranspiration is maximum in May (11.34 mm/h) while lowest in December (3.24 mm/h).

From edaphic point of view, a wide range of soils occur in this region. Right from laterite to red soil and to black cotton soils occur in this area. The red and black cotton soils are sodic in nature and these show overall deficiency of potassium. The water holding capacity also varies from 30.26% to 76.18% (from unpublished data).The soils under irrigation, especially black cotton soils are rich in humus.

Taxonomic survey of this region showed that some weed populations dominate this area either in cropping season i.e. “in season” or after cropping season i.e. “off the season” (Murumkar and Magdum, 1991) or throughout the year. After preliminary survey of weed populations of this region, *Aristolochia bracteolata* Lam. is selected as an experimental material which is a dominant weed of this region.

The common dominant weed, *Aristolochia bracteolata* Lam**.** is commonly known as worm killer in English. It belongs to family Aristolochiaceae. This Plant is one of the worst weeds of black cotton soil. It has been found to occur also in dry, irrigated, loamy and clayey soils, though not to a great extent. It is reported to be poisonous to man and livestock as it possesses insecticidal or insect repellent properties due to the presence of Aristolochic acid (Watt and Breyer-Brandwijk, 1962).The plant is reputed as indigenous medicine for its bitter, purgative and anthelmintic properties.

The preliminary floristic analysis has revealed that *Aristolochia bracteolata* Lam. is a prominent dominant weed of fallow land. As this weed is more common in winter and summer season, it certainly attracts the attention. The occurrence of this weed in a variety of habitats and soil types reveals its uniqueness which has prompted us to understand this weed in more elaborative way. Interestingly, earlier Deshmukh and Murumkar, (1996) have reported occurrence of Crassulacean Acid Metabolism in *A. bracteolata* Lam. for the first time.

Keeping this in mind, we felt it worthwhile to study the ecophysiology of *Aristolochia bracteolata* Lam. as this plant is growing well even under unfavourable conditions like extreme hot summer of this region. It was also essential to probe into various physiological processes and microbial studies in this weed.

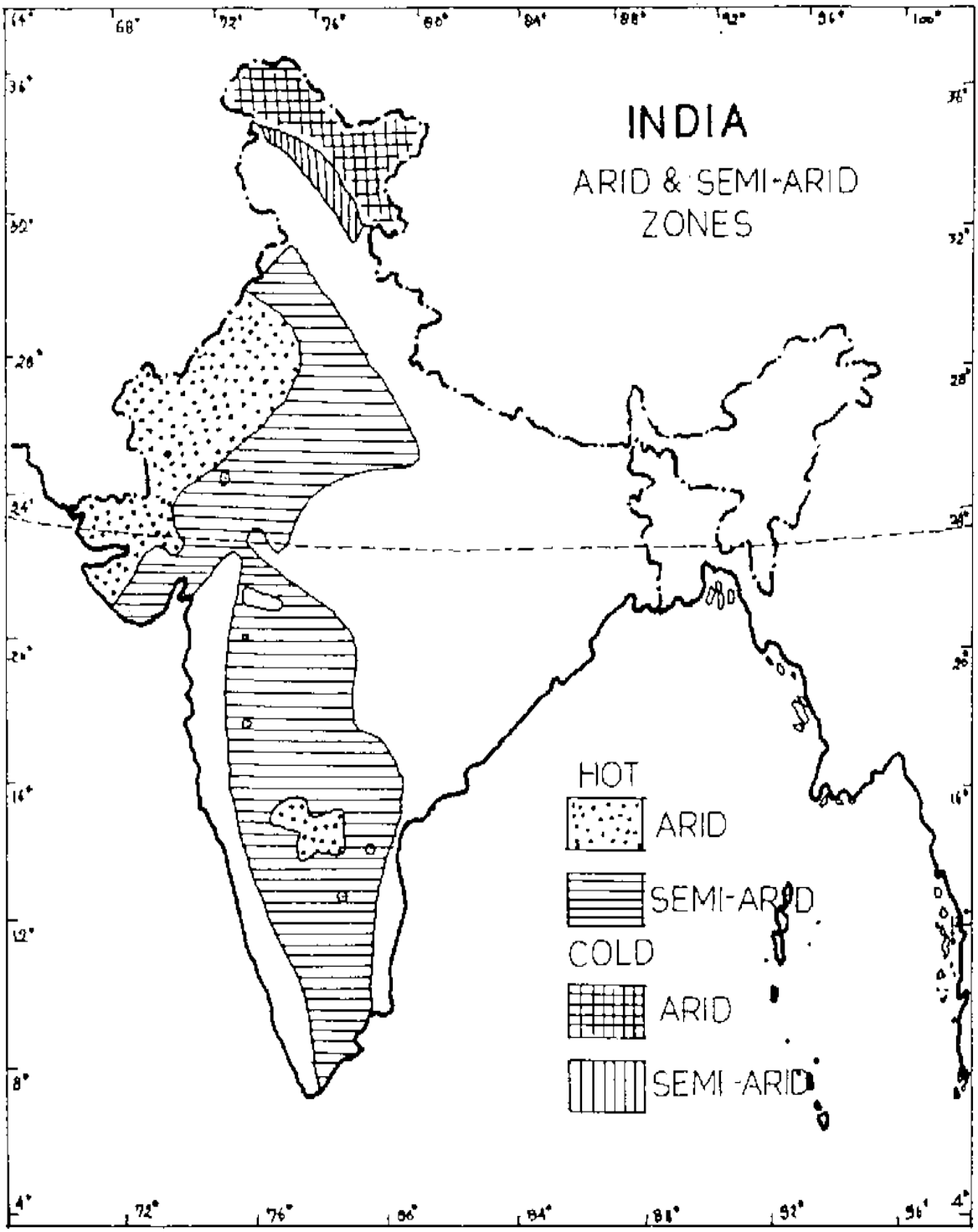


Figure 1: Map of India Showing arid and Semi –arid regions

(Courtsey S. P. Sen,1994)

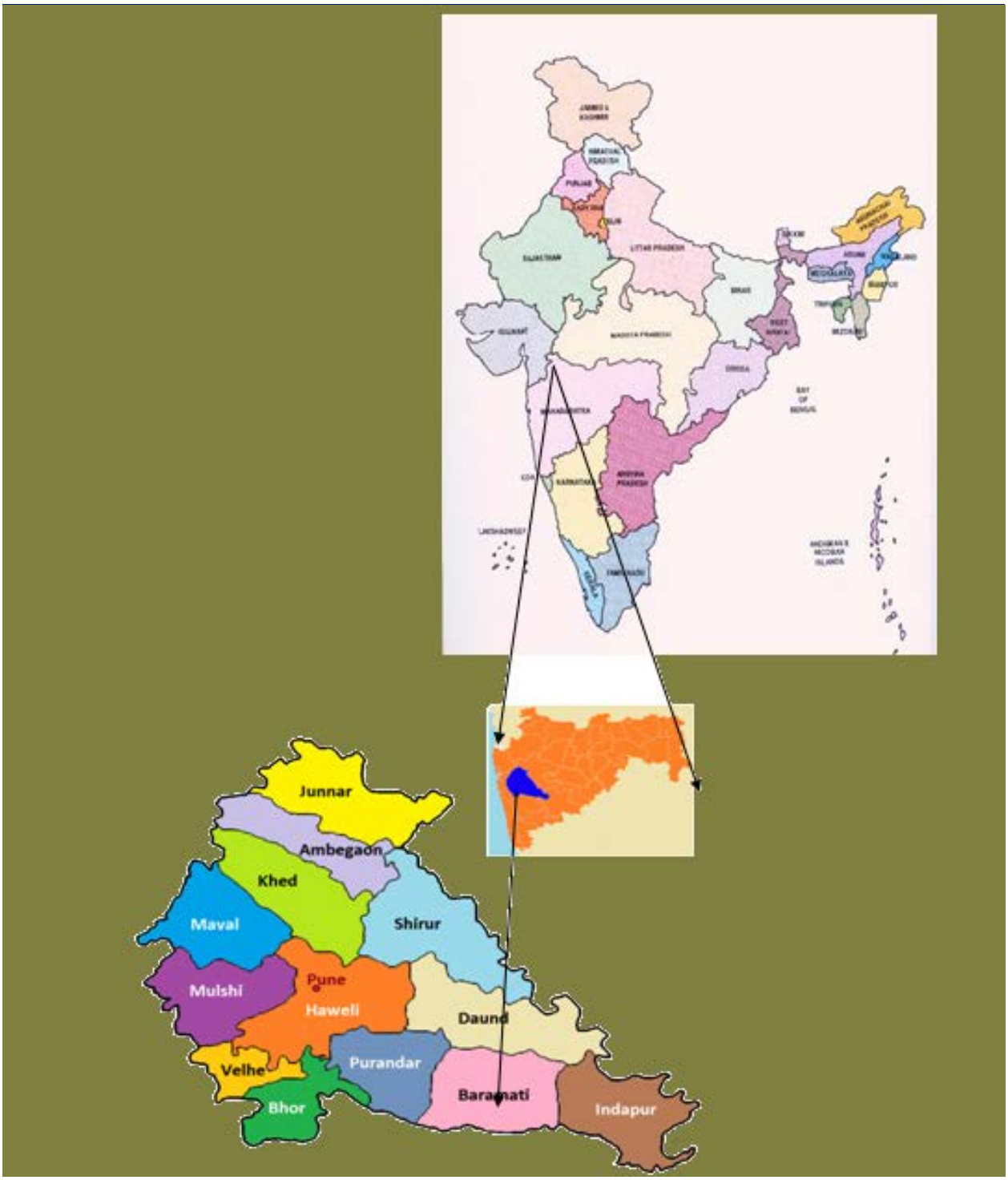


Figure 2: Study area : Baramati Region

In present study, this plant is studied to know its life cycle pattern, habitat, vegetative characters and yield output. Work is further extended to study ecophysiology of this plant to understand it's success of survival in arid conditions and this involves study of germination behaviour, growth pattern, water status, the mode of photosynthesis, metabolism of organic solutes as well as role of secondary metabolites like free proline, total polyphenols etc. With these physiological parameters, the probable cause of its success as a dominant weed in this area is being found out.

Aristolochic acid is important component of this plant. Isolation and characterisation of aristolochic acid from *Aristolochia bracteolata* Lam. is of prime importance. Aristolochic acid is characterised with use of advanced techniques like GCMS, HPLC, IR spectroscopy, NMR Spectroscopy and X-ray crystallography.

This weed is found in field in almost all seasons. This also thrives well in hot summer season and stands alone as a dominant weed in the field. In order to evaluate its success towards drought resistance the study is further extended to analyse water stress. The growth pattern, mineral nutrition, photosynthesis, enzymology and metabolism have been studied to understand *modus operendi* in this weed towards water stress.

On the other hand, as this weed is known for medicinal value the, study is further extended to focus more light on production and utilization potential of some novel components of medicinal importance because now a days there is an increasing interest worldwide in herbal medicines. Such studies encompass investigation of physiological parameters, analysis, pharmacological properties of bioactive compounds and their ability to treat various diseases. In present study, *Aristolochia bracteolata* Lam. is also screened for its antibacterial and antifungal potency as only few reports are available, still so far.

The overview of this research is presented here in the form of thesis. The Thesis is divided into five chapters. The current chapter I is the Introduction which is emphasizing present weed science scenario and the work done so far in different parts of the world. There is strong requirement for studies in various areas for better understanding of crop weed relationship and the strategies adapted. The information gathered clearly justifies the need of present study.

In order to understand the work done so far in this weed *Aristolochia bracteolata* Lam., an elaborative Review of Literature is presented in chapter II. More emphasis has been given on various taxonomical, systematic, ecological, genetic as well as physiological aspects. Looking towards the available data the need of more research in this weed can be clearly visualized.

The methodology followed in present investigation is presented in detail in chapter-III. It includes basic frame work and design of the research work undertaken, different techniques and methodologies applied, and also statistical treatment for the findings. Advanced techniques like as HPLC and GC-MS, XRD also have been applied by seeking help of other laboratories as per the need of the experimentation for clear and objective understanding.

The findings of present investigation are presented in relevant logical manner and the same are discussed in light of relevant literature and this forms the content of Chapter –IV i.e. Results and discussion. In the last chapter V of the thesis, the significant findings are briefly summarised in the form of Summary and Conclusion. The literature sited in the thesis is neatly presented in bibliography part of the thesis at the end.

CHAPTER II

REVIEW OF LITERATURE

Weeds are as old as agriculture itself. They form one of the most deleterious and expensive component in agriculture. It is of scientific interest that man has failed to control this persistent problem. On the other hand, he has seen them helplessly to create problems by their growing, spreading and disseminating seeds freely.

About 30,000 weed species are widely distributed in the world; 1800 of which cause crop yield losses every year that make up about 9.7% of total crop production. Weed control has always been an important aspect of environmental protection. Over the past century, chemical herbicides have been effectively employed to control various weeds. However, they have caused many serious side-effects such as herbicide resistant weed populations, reduction of soil and water quality, and detrimental effects of herbicide residues on non-target organisms etc. (Te Beest and Templeton, 1985). Over the last decade, weed science research has shifted it's focus from herbicide discovery and its physiology to the study of weed biology and ecology. An overriding question is what makes a species a successful agricultural weed? This central question has been tackled from a variety of angles, identifying and characterizing attributes contributing to weed success. Now a day's tools of physiology, molecular biology and genomic research are being utilized increasingly in these efforts.

In India, weeds in general have remained neglected for many decades, even though they affect economy of agriculture. Literature reveals that, the work on weed *Aristolochia bracteolata* Lam. in country and outside as well has progressed along the following lines like weed survey, physiology, phytochemistry, pharmacognosy, ecology, pathology, and microbiology etc.

Aristolochia is an important genus of order Piperales from the sub-family Aristolochoideae of family Aristolochiaceae and represented by 500 species (Kiew, 1999).

Etymology

The name *Aristolochia* (meaning the best delivery or birth) is thought to be of ancient Greek origin and reflects centuries of use in obstetrics *aristos* meaning "best" or, originally, "most fitting" and *lochia* which means "delivery." This is due to its original use to expel the placenta after childbirth. Other traditional uses include

treatment for snakebite, scorpion stings, fever, infection, diarrhea, and inflammation (Arlt *et al.,* 2002, Jiménez - Ferrer *et al.,* 2005). *Aristolochia bracteolata* Lam. is commonly known as English - Wormkiller, bracteated Birthwort; Hindi- Gandan, Kidmari; Sanskrit - Dhomapatra, Gridhrani, Kitkaha, Kitari; Gujrati – Kidmari; Marathi - Gandhan, Gandhati; Kanada - Kattackirubangida; Malayalam - Aduthinnapala; Orya - Paniri; Tamil – Adulthinnapalai; Telgu – Gadugagudupa (Kirtikar *et al.*,2005).

Origin and phylogenetic information

Aristolochiaceae family has been recently the subject of more attention because of the suggestions that these basal angiosperms are phylogenetically close to the divergence of monocots from dicots. Aristolochiaceae was traditionally placed in subclass Magnoliidae and thought to be related to woody members of the subclass Annonales; however, the first morphological cladistic analyses of basal angiosperms supported relationships of the family with other predominately herbaceous magnoliids (Piperales, Nymphaeales, Lactoridaceae) and the monocots. Subsequent phylogenetic studies supported relationships of Aristolochiaceae with more restricted set of paleoherbs (Lactoridaceae, Piperales, monocots), as well as Magnoliales, Laurales, and Chloranthaceae (Kelly and Gonzalez, 2003).

Phylogeny of Aristolochiaceae based on parsimony, likelihood and Bayesian analyses of trn L- trn F sequences has been done to resolve position of Aristolochiaceae in Piperales (Neinhuis, 2005). This study addresses the overall generic relationships in Aristolochiaceae and its position in Piperales based on dense taxon sampling and sequence data from the plastid trnL-F region. All members of Aristolochiaceae have anatropous, bitegmic, crassinucellate, endostomic ovules with a few exceptions (González, 2003). Razzak *et al.*,(1992) studied pollination biology of *Aristolochia bracteolata* Lam. and reported that it is partially Protogynous. The flower exibits fly-trapping mechanism while pollen germination takes place within the dehisced anthers and on perianth parts.

Geographic Distribution

Aristolochia is the genus of shrubs, or rhizomatous perennial herbs, often twinning, mainly distributed throughout the tropics, but some species occur in warmer temperate regions also. The greatest diversity in *Aristolochia* species is found in Central and South America (USDA, 2010). In Malesian region 28 species have been

reported (Anonymous,1948). *Aristolochia tagala* Cham. has the largest area of distribution occurring from India and China, throughout South - East Asia to Australia. (Kiew,1999).

Species of *Aristolochia* usually occurs scattered, often in primary forests, but some species are also found in secondary forest and scrub vegetation. The genus *Aristolochia* consists of species of herbaceous perennials, undershrubs or shrubs bearing essential oils and is widespread across tropical Asia, Africa and South America. Most European and tropical species of *Aristolochia* are believed to be pollinated by small flies attracted to the flowers by the foetid odours and purple-brown colour. Flies enter the flower when the stigmas are receptive and are trapped until after the anthers dehisce (Solereder, 1889). More than 30 *Aristolochia* species are native to the United States and occur in almost all states (USDA 2010).

Indian Status

About 15 species of *Aristolochia* occur in India of which three namely *A. bracteolata* Lam., *A. indica* Linn. and *A. tagala* Cham. are useful in medicine (Santapau and Henry, 1973). The presence of Aristolochic acid, a nitrophenanthrene compound with tumour inhibiting properties, appears to be characteristic of the genus (NTP, 2008). Among these, *Aristolochia bracteolata* Lam. is most dominant in India and globally distributed in Tropical Africa, Arabia, Sri Lanka, and Pakistan. Within India, it occurs in northern and central India from Haryana to West Bengal and southwards to Tamil Nadu and Kerala. It is one of the dominant weed species of Maharashtra often found in dry regions (Murumkar, 1993).

Plant Description

Aristolochia bracteolata Lam. is a slender, decumbent, glaborous perennial with 30-45 cm long. It is found to be distributed in hedges in the plains of Northern India from Haryana and Utter Pradesh and southwards to peninsular India up to Maharashtra and Andhra Pradesh. Leaves are varying variably in size and shape, reniform or broadly ovate, crenulate, undulate, long petioled. Flowers borne solitary, with perianth 2.2 - 5.0 cm long with subglobose base. Perianth tube cylindrical with trumpet shaped mouth and linear, dark purple coloured lip presence of glandular hairs within. Capsules are oblong-ellipsoid, 12 ribbed, glaborous. Seeds are deltoid with cordate base, rugose black on one side and whitish on the other, compressed (Wealth of India, 1982).

Cultivation

Several species of *Aristolochia* are grown as ornamentals chiefly on account of their remarkable, beautiful and unusually shaped flowers. The plants bloom during summer as well as the monsoon, and bear a succession of highly coloured, mostly foetid flowers of variable size and shape. The individual flowers may last for a couple of days in sheltered positions. Although erroneously called 'Fly Catchers' i.e. *Aristolochias* are not insectivorous, but are insect pollinated plants i.e. entomophilous.

The ornamental plant requires plenty of sun, protection from hot winds and mulch in the plains for overwintering. Garden soil mixed with leaf moulds and cowdung is desirable for cultivation of *Aristolochias.* Propagation is usually by seeds or by layering and cutting taken from well matured wood in early spring or at pruning time (Bor and Raizada, 1954).

Germination

Reports of germination studies are meagre. Only a few reports are available stating that *Aristolochia* seeds exhibit seed dormancy and highlighted various treatments for breaking seed dormancy. Maekawa, (2010) reported that presence of light and temperatures at 25^0C and 30^0C favoured seed germination and normal seedling formation in *A. esperranzae* Whereas treatment of long period of cold stratifying temperatures was required for growth of underdeveloped embryos and seed germination in *A.calfornica*, *A Macrophylla*, *A.tomentosa* and *A.manshurensis* (Christopher, 2005).

Anatomy

Nair and Narayanan (1962) studied the Nodal and floral anatomy of Aristolochiaceae and reported that in *A. bracteolata* Lam. the vascular system of the axis, immediately below the node, consists of nine large, widely separated collateral vascular bundles. All of them enter the lamina to display the characteristic palmate venation. The leaves in *A.bracteolata* Lam. are cordate and palmately veined. The stomata are present only on the lower surface and are of the Ranunculaceous type (Metcalfe and Chalk, 1950). Foliar stomatal development in three species of *Aristolochia* has been studied by Philip (1983).

Conservation Status

All species of *Aristolochia* are included in list A of Schedule I of the Export (Trade) Control Order, 1988 as the export of this plant and it's derivatives is banned. Collection of plant material from Reserved Forests is restricted under the Indian Forest Act. Low Risk-Least Concerned status has been assigned to the *Aristolochia bracteolata* Lam. in red list medicinal plant species. Kayode and Ogunleye (2008) included *A.bracteolata* Lam. in rare category in his "Checklist and Status of Plant Species" which is used as spice in Kaduna State of Nigeria as is rich in natural products like Alkaloids, magnoflorine, aristolochine, aristolochic acid.

Survey and Ethnobotanical Uses

Members of *Aristolochia* has been used since ancient times in traditional herbal medicines in many parts of the world, and Aristolochic acid it contains has been reported to have antibacterial, antiviral, antifungal, and antitumor effects (Kupchan and Doskotch 1962, Zhang *et al.*,2004). As contemporary medicine, *Aristolochia* plant extracts have been used in therapies of arthritis, gout, rheumatism, and festering wounds. However, its use was discontinued in Germany and other countries after the carcinogenic and mutagenic properties of aristolochic acids were first reported in the early 1980s by Arlt *et al.* (2002). Saha *et al.* (1961) presented a list of 277 medicinal plants possessing abortificient, emmenagogue as well as ecobolic properties, and *Aristolochia bracteolata* Lam. is one of them.

Singh *et al.*(1973) enlisted *Aristolochia bracteolata* Lam. as dominant terrestrial weed in his work on weed flora of Andhra Pradesh. Sankaranarayanan *et al.*,(2010) reported that this plant was used by traditional users from Villupuram district of Tamil Nadu for Ethnobotanical study of medicinal plants. The plant is used for treating foul ulcers, skin diseases etc. Leaves of this plant mixed with castor oil are applied externally to children's legs in obstinate cases of eczema, while roots used to treat male sterility. Masilamani (1997) reported that *Aristolochia bracteolata* Lam. is also practiced for snake-bite by Gounda Tribals of Tamilnadu. Dixit and Pandey (1984) reported that this plant is used as a folk medicine in Zansi and Lalitpur sections of Bundelkhand, Utter Pradesh. Earlier, Jamwal and Anand (1962) also reported that juice of whole plant was applied to ulcers. In Maharashtra also, this plant is used as a local medicine for curing ulcers (Deokule and Magdum,1992).

Conservation and Biotechnology

Rao *et al.,* (2005) reported occurrence of this weed in Ahmednagar district in Maharashtra and tried for its *ex-citu* conservation.

Experiments on *in-vitro* propagation have been successful for *A.bracteolata* Lam. in India. Callus formation was observed from young leaves and roots placed on Murashige and Skoog medium supplemented with Kinetin, Naphthalene Acetic Acid and Indole Acetic Acid. Roots were initiated from callus when the concentrations of kinetin and indole acetic acid were increased. From a single nodal segment, 1-4 shoots were raised and grew 5-6 cm tall within 30 days (Gbadamosi, 2012). Whereas Sahaya (2011), standardized *in vitro* protocol for mass propagation of *A.bracteolata* Lam.using internodal segments of explants on Murashige and Skoog's basal medium varying the concentrations of auxin and cytokinin. Similarly work of Sebastinraj (2011) emphasized the responses achieved in terms of direct regeneration from meristematic axillary bud explants of *A.bracteolata* Lam. *in-vitro* studies.

Medicinal uses

As early as in 1936, Kirtikar and Basu had reported this plant as bitter, purgative, anthelmintic and for destroying the larvae of insects. The root decoction is employed to expel roundworms and administered as a dry powder or infusion to increase uterine contractions. However, laboratory trials have failed to confirm this activity. It is also reported as an abortifacient, however, pharmacological trials have not accepted this claim. The bruised leaves mixed with castor oil are applied externally in eczema. The oil is also applied to navals of childrens in colic and also given internally with castor oil. Jamwal and Anand (1962) had reported this plant also during preliminary screening for some abortifacient indigenous plants. Other reports for traditional uses include treatment for snakebite, scorpion stings, fever, infection, diarrhoea and inflammation (Arlt *et al.*,2002).

Aristolochia is reported to be poisonous to man as well as live stock. It also possess insecticidal as well as insect repellent properties due to the presence of aristolochic acid (Chopra *et al.*,1956). In the indigenous system of medicine, the decoction of leaves is used for treating skin diseases and rheumatism (Anjaria *et al.*,2002). It has been used for treatment of roundworm infection (Nadkarni and Nadkarni, 1954). The root and leaves of this weed *A. bracteolata Lam* are bitter,

acrid, thermogenic, cathartic, anti-inflammatory, antibacterial and useful in constipation, foul ulcers, eczema and intermittent fevers (Warrier *et al.*, 1994; Joy *et al.*,1998).

In Rajasthan, the seeds are employed for softening hair. The seeds yield yellowish green non-drying oil on petroleum ether extraction. Zarroug *et al.,* (1988) observed that out of eight plants studied only *Aristolochia bracteolata* Lam. extracts showed larvicidal activity.

Phytochemistry

Since the Greek-Roman period, Aristolochic acid, a constituent of *Aristolochia* species, has been used for medicinal purposes (Pezzuto *et al.*,1998). Several investigations have been carried to evaluate the role of aristolochic acid in medicine in different species of *Aristolochia* namely *A. elegans* (Shi *et al., 2004), A. albida* (Lagide *et al.*,1993). *A. papillaris* (Lemos *et al.,* 1993) *A. mollissima* (Yu *et al.,* 2007), *A. tringularis* (Rucker *et al,* 1981), *A. fangchi* (Martinez *et al.,* 2002), *A. constricta* (Rastrelli *et al.,* 1997), *A. cucurbitifolia* (Wu *et al.,* 1999), *A. rodriguesii* (Correa *et al.,* 1998), *A. pubescens* (Nascimento and Lopes, 2003), *A. anguicida* (Gaitan *et al.,* 2002), *A. malmeana* (Messiano *et al.,* 2008), *A. cymbifera* (Leitao *et al.,* 1992). *A. hamissonis* (Bomm *et al.,* 1999), *A. ringens* (Larrahondo and Acevedo,1990).

The taproot of *A. bracteolata* Lam. bears gradually tapering branched roots and numerous fibrous rootlets. The root has no characteristic odour but has a nauseously bitter taste. The root powder exhibits a violet fluorescence in ultraviolet light when mounted on nitrocellulose. The root contains aristolochic acid, potassium chloride and nitrate in large amounts (Prasad and Vohra, 1960).

Extensive investigations on the terpenoides of *Aristolochia* genus have been carried out by Tian –shung *et al.,* (2004). Also Pacheco (1981, 2007, 2009) presents a compilation of large number of terpenoidcompounds, particularly diterpenes and the 13C-NMR data of 57 diterpenoids isolated from *Aristolochia* species. The compounds are arranged skeletonwise in each section, according to their structures, i.e., clerodane, labdane, and kaurane derivatives. Tian *et al.,* (2011) worked on Chemical constituents and Pharmacology of the *Aristolochia* species and concluded that aristolochic acid concerns with nephrotoxicity. Heynauer (1960) made

chemotaxonomic studies to evaluate taxonomic position of Aristolochiaceae in dicots. Lopes and Bolzani (1988) had reported occurrence of lignans and diterpens from three species of *Aristolochia* including *A. bracteolata* Lam.

This plant contains several alkaloids like Aristolochic acid, aristolic acid, leutin and many other compounds (Anonymous, 1985). Saha *et al.*,(1961) studied chemical constituents of *Aristolochia* plants and found that leaves and fruits of *Aristolochia bracteolata* Lam. showed presence of ceryl alcohol (Leaves, 0.38%, fruits, 0.12%), β –sitosterol and potassium chloride, besides alcoholic acid. Prasad and Vohra (1960) worked on pharmacognostic activity in *A.bracteolata* Lam. They showed that root powder exhibited violet fluoroscence in UV light when mounted in Nitrocellulose. Isolation and characterisation of eupamatenoid derivatives was carried out by Enciquez *et al.*,(1984). Whole plant *A.bracteolata* Lam yielded the aporphine alkaloid N. Acetylnoferine and aristolactam. (Chakravarthy *et al.*,1988).

Screening of this plant for alkaloid content was done by Kokai, (1983) and Zhang *et al.*,(1987). Magnoflorine and aristolochic acid –I were isolated from the seeds and their pharmacologic actions studied in *Aristolochia bracteolata* Lam. (Tahir and Kamal, 1991). Hussein (1970), isolated an alkaloid magnoflorine and two acidic compounds aristolochic acid and aristored in pure crystalline form. In addition, two unidentified quaternary alkaloids have been separated in small amounts in a crude form. Hassan *et al.,* (2004) determined phytochemical constituents and fatty acid composition of the refined seed oil of *Aristolochia bracteolata* Lam. The results showed that seed oil was rich in linoleic acid (39.0%) and oleic acid (32.8%).Trace quantities of epoxy and hydroxyl fatty acids were also present in the seed oil. Kalpana devi *et al.,* (2011) screened this plant for phytochemical analysis and biological property findings.

Allelopathy:

Deokule and Avachar (2006) reported allellopathic influence of *Aristolochia bracteolata* Lam. on seedling germination of *Cucumis sativus* Linn. Allelopathic effect of aqueous extracts of *Artistolochia esperanzae* O. Kuntze on development of *Sesamum indicum* L. seedlings was studied by Gatti *et al*.(2010). They observed significant changes in germination behavior and seedling growth. Greatest inhibition was recorded in primary root length, number of secondary roots and reduction in size

of root xylem cells of *Sesamum* seedlings when treated with root extracts of *A. esperanze*.

Pharmacology

Anti-Inflammatory

Shiwaikar and Somshekhar (2003) studied antioxidant effect of the ethanol extract from *Aristolochia bracteolata* Lam. leaves using nitric oxide and 1,1-diphenyl-2 picryl hydrazyl (DPPH)-induced free radical assay methods which showed free radical scavenging activity, thereby supporting its anti inflammatory properties.

Anti-bacterial

Negi (2003) reported antibacterial activity in *Aristolochia bracteolata* Lam. root extracts. In another study, Aristolochic acid from roots of *Aristolochia bracteolata* Lam. was found to be good antimicrobial agent (Angalaparameswari *et al.,* 2000). Manikandar *et al.,* (2006) studied antibacterial activity in leaf extracts of *A. bracteolata* Lam. The species of *Aristolochia* like in *Aristolochia heterophylla* and *Aristolochia kaempferi* also showed significant antibacterial activity of distilled water and chloroform extracts (Wu *et al.,* 2000). Nagaragan *et al.*,(2009) worked on comparative antimicrobial efficacy of ethyl acetate and ethanol leaf extracts of *A.bracteolata* Lam. Root, stem and leaf extracts of *A. bracteolata* Lam. was tested against three clinically isolated bacteria (Kadam *et al.,* 2012). They observed maximum antibacterial activity in root extracts.

Antifungal

Ramasubramania and Niranjanbabu (2011) screened Aristolochia *bracteolata* Lam. for phytochemical, pharmacognosical and antifungal activity and concluded that even minimum concentrations of ethanolic extract were effective against *Trichiophyton rubrum* and *Microsporum canis* responsible for ringworm infection. Kavita and Nirmaladevi (2007) also observed positive reports of different extracts of *A.bracteolata* Lam. against selected fungi.

Antiplasmodial

Tahir (1999) studied antiplasmodial activity of this plant in selected Sudanase medicinal plants with special emphasis on *A.nilotica*. This plant is also used as insect repellent against flies and maggots (Secoy and Smith, 1983). The extract of *Aristolochia bractelolata* Lam. exhibited inhibitory activity against *Plasmodium falciparum* strain. *Aristolochia bracteolata* Lam. was found to have a trypanocidal effect against *Trypanosoma evansi* (Samia *et al*., 2006).

Herbicide

Myakado *et al.*,(1986) reported Aristolochic acid from this plant extract having herbicidal property. Saxena (1979) concluded Aristolochic acid an insect chemosterilant from *A. bracteolata* Lam. Thirumal *et al*.,(2012) gave detailed survey of literature of pharmacognosical, ehnobotanical, traditional as well as phytochemical and pharmacological reports on *A. bracteolata* Lam. Harbone (1988) reported toxicity of *A.bracteolata* Lam. due to supposed anthelmintic activity and trypanocidal effect. Zarrong *et al.* (1988) observed that out of eight plants studied only *A.bracteolata* Lam. showed slight larvicidal activity. Comparative lipid peroxidation inhibition of the two *Aristolochia* species *viz A. indica* and *A.bracteolata* was reported by Vijaykumar *et al.*,(2003).

Antioxidant

Tiruganasampadan *et al.*,(2008) observed that antioxidant properties of some medicinal Aristolochiaceae species including *A. bracteolata* Lam. Shirwaikar *et al.*, (2003) observed that this plant showed positive effect on wound healing with significant increase in the level of antioxidant enzymes like super oxide dismutase and catalase in the granuloma tissue.

Physiology

Aristolochia bracteolata Lam. is a dominant weed of hot semiarid region persisting even in hot summer (Murumkar and Magdum, 1991). Occurrence of Crassulacean Acid Metabolism in leaves of *A. bracteolata* Lam. has been reported for first time by Murumkar and Deshmukh (1996). While in support of this data, Facultative CAM nature is exhibited in this plant (Kadam *et al.*,2012)

Aristolochic acid

Aristolochic acid is characteristic feature of family aristolochiaceae and *Aristolochia, Asarum* spp. (Guan *et al.,* 2005). It also has differential effects on human body. Some claim stimulation of white blood cell activity which gives remarkable healing powers, but is carcinogenic and damaging to the kidneys (Chevallier, 1996).

Abdelgadir *et al.* (2011) have worked on isolation, characterisation and quantification of aristolochic acid in *A. bracteolata* Lam. Jou *et al., (*2004) studied analogues of aristolochic acid and aristolactam in *Aristolochia* by HPLC, while Simultaneous determination of five aristolochic acids and two aristolactams in *Aristolochia* plants by HPLC has been confirmed by Zhang *et al.* (2006).

Rapid determination of Aristolochic acid I and II in some medicinal plants by HPLC has been given by Wei *et al.* (2004). Zhai *et al.* (2006) described method for separation and determination of Aristolochic acid in herbal medicines by microemulsion electrokinetic chromatography. The quantitative analysis of aristolochiaceae determined that all the species contain aristolic acid (Hashimoto *et al*., 1999). It was reported that *Aristolochia* produced intestinal nephritis caused by aristolic acid during treatment (Mengs and Stozen, 1993).

Biosynthesis of Aristolochic acid.

Biosynthesis of Aristolochic acid is illustrated by Sharma *et al.,* (1982). An important intermediate in the biosynthetic pathways of Aristolochic acid is the benzylisoquinoline, which can be formed from tyrosine or a similar biochemical equivalent (Schutte, 1967). The incorporation of tyrosine 3, 4 dihydroxyl alanine nororientaline, orientaline(I), prestephaine(II) and Stephanine (III) into aric acid (IV) in *A. bracteolata* Lam showed that specific utilization of (I). Mukhopadhyay *et al.,* (1983) in their study on *Aristolochia* showed that conversion of Aristolochic acid I to Aristolochic acid is one step removal of nitro group.

Cytology

There are few reports on cytology of *Aristolochia.* In *Aristolochia* there are species with (n=7), aneuploid to (n=4) and polyploids (Raven, 1975). Somatic chromosomes of five species of *Aristolochia* including *A. indica* were investigated by Sharma and Verma (1959) and they concluded that *A. indica*, having the lowest number of diploid chromosome (2n=12) with only one pair of chromosome and secondary constrictions, probably represents the most primitive species of the five studied. Sanjappa (1979) reported that *Aristolochia bracteolata* Lam. is with gametophytic number Six.

Through screening of available literature on *A. bracteolata* Lam. depicted that this weed serves as local medicine and popular remedy among the various ethenic groups. It is interesting to note that crude as well as organic extracts of root, stem and leaves have been screened for pharmacological activities. However, the isolated principle needs to evaluate in clinical trials for the molecular mechanism of action in lead molecules from natural resources. As global scenario is now changing towards the use of modern drugs from medicinal plants. for treatment of various diseases. Nevertheless, it must be admitted that in view of limited physiological studies in *Aristolochia bracteolata* Lam. the findings reported in the present investigation may

give broad idea about the working of important physiological processes like mineral nutrition and photosynthesis in this plant under unfavourable environmental conditions despite the possibility that these important factors are determining the response to arid conditions.

It can be seen from the forgoing account that most of the work done in *Aristolochia bracteolata* Lam. is in the field of pharmacology and chemical investigation. Although, antimicrobial nature is well documented in the above studies much is to be understood about the process like photosynthesis, nitrogen metabolism, effect of water stress as this is very common dominant weed in this arid region and blooms fully in summer season also.

CHAPTER III

MATERIAL AND METHODS

A) Habitat Analysis

i) Study area -

Baramati (18^0, 8' N and 75^0, 7' E) is a taluka place of Pune district in Maharashtra, India. This region is a distinct semi-arid zone because of the rain shadow effect of Western Ghats. This place has mean annual maximum temperature 33.47^0C and minimum 20.03 ± 2.00^0C (Murumkar, 1993). Highest daily temperature is recorded during May (mean max.36.95^0C) and lowest in December (mean max.17.07^0C). During summer, especially in April and May, daily maximum temperature many a times shoots up beyond 40^0C. On an average this region is dry with almost 7 months of dry hot period.

The relative humidity is highest during months of September (83.69%) while the lowest recorded in April (55.11%).The rate of evapotranspiration is maximum in May (11.34 mm/h) while lowest in December (3.24 mm/h).

From edaphic point of view, a wide range of soils occur in this region. Right from laterite to red soil and to black cotton soils occur in this area. The red and black cotton soils are sodic in nature and these show overall deficiency of potassium. The water holding capacity also varies from 30.26% to 76.18% (from unpublished data).The soils under irrigation, especially black cotton soils are rich in humus.

ii) Studies on population -

The vegetation of *Aristolochia bracteolata* Lam. weed was surveyed in years 2011-12 and 2012-13. Fully mature healthy plants occurring in cultivated and open fields were collected from the quadrates (**1x1 m**) using RBD design. The collected plant specimens were identified and confirmed with the help of officials from Botanical Survey of India, West Zone office, Pune.

In the present investigation, efforts were made to collect the plant material from areas around Baramati. Frequent visits were arranged to collect the plant material. Herbarium specimens were prepared, identified and authenticated with the help of BSI, Pune. Finally herbarium specimens were deposited in P.G. Research centre of T. C. College, Baramati. During collection of these specimens the pattern of

vegetative growth, its flowering, fruiting and seed setting period was recorded throughout the year and used for further analysis. The survey conducted from different sites nearby Baramati. The sites were selected randomly encompassing all habitats to cover the maximum area.

iii) **Rhizosphere soil analysis** -

a) **Collection of soil samples** -

Soil samples associated with *A. bracteolata* Lam. from agricultural and open fields were brought to laboratory and spread on a paper. Stones, gravels and other coarse residues were removed. Larger pieces of soil were broken by hand. The air dried soil samples were crushed in mortar and pestle and sieved through 2 mm nylon sieve. These samples were used further for analysis of various physical and chemical characters.

b) **Physical Characters** -

i) pH-

One gram of soil sample collected was homogenized in mortar with pestle in 25 ml D.W. and from the filtrate pH was determined using Elico Digital pH meter (Model Li 120).

ii) Electrical Conductivity(E.C.) –

From the above extract, electrical conductivity was measured with the help of Conductivity Bridge (Elico Model cm.827).

iii) Organic Carbon -

The standard wet chemistry technique for the sample extraction involving the rapid dichromate oxidation of organic matter was employed as per Walkley-Black (1934). In this procedure, potassium dichromate ($K_2Cr_2O_7$) and conc. H_2SO_4 were added to 1.0 g of the soil sample. The solution is swirled and allowed to cool. Prior to adding water to halt the reaction, Orthophosphate, H_3PO_4 is added to the digestive mixture to eliminate interferences from the ferric (Fe^{3+}) iron that may be present in the sample.

One gram of the prepared soil sample was taken in a 500 ml conical flask. To this, 10 ml of 0.1667 M $K_2Cr_2O_7$ solution and 20 ml of concentrated H_2SO_4 containing Ag_2SO_4 was added, mixed thoroughly and finally allowed the reaction to complete for 30 minutes. The Reaction mixture was diluted with 200 ml with distilled water and 10 ml of H_3PO_4 and then to this 10 ml of NaF solution and 2 ml of diphenylamine indicator was added. The final solution was titrated with standard 0.5M $FeSO_4$ solution till a brilliant green color was obtained. Simultaneously, a blank without sample was also run.

The percentage of organic C is calculated using following formula

Organic Carbon = 3 (S-T) / S

where:

S = ml of $FeSO_4$ solution required for blank.

T = ml of $FeSO_4$ solution required for soil sample.

c**. Chemical Characters -**

i) Total Nitrogen -

For estimation of the available N content of the soil, alkaline permanganate method of Bremner (1960) was followed. Oven dried, powdered 0.5 g of soil sample was taken in Kjeldahl flask with a pinch of microsalt (200 g K_2SO_4+ 5g $CuSO_4$, dehydrated) and to it 5 ml H_2SO_4 (1:1) was added. Few glass beads were added to avoid bumping and the material was digested on low flame. After complete digestion a faint yellow solution was obtained which was cooled to room temperature, transferred to volumetric flask and diluted to 100 ml with distilled water.

In very clean Nessler's tube, 1 ml of soil extract and different concentrations of standard ammonium sulphate solution (0.236 g Ammonium sulphate dissolved in water and few drops of H_2SO_4 are added and the volume is made 1000 ml. This solution contains 0.05 mg of nitrogen per ml) are taken. In control tube 1 ml distilled water was taken. To this, 1 drop 8% KH_2SO_4 is added and volume is made 35 ml with distilled water. To this, 15 ml Nessler's reagent is added (Reagent A: 7 g KI+ 10 g HgI_2 in 40 ml distilled water, Reagent B: 10 g NaOH in 50 ml water, A and B are to be mixed in proportion of 4:5 at the time of estimation). After 15 minutes, the absorbance of the

chromophore developed is read at 520 nm using ELICO spectrophotometer. Nitrogen value is calculated from the standard curve. Values are expressed as mg/g dry matter.

ii. Phosphorous -

For estimation of P^{5+} the method of Chapman and Pratt (1961) was followed. Here, phosphorus reacts with 'Molybdate Vanadate reagent' to give yellow colour complex. By estimating colorometrically the intensity of the colour developed and by comparing it with the colour intensity of known standards, phosphorus content was estimated.

To 1 ml of acid digest in a test tube, 2 ml of 2 N HNO_3 was added with 1 ml Molybdate Vanadate reagent (1.25 g of ammonium molybdate in 500 ml 1 N HNO_3 + 25 g of ammonium vanadate in 500 ml distilled water mixed in equal volumes) and volume was made to 10 ml with distilled water. The ingredients were mixed well and allowed to react for 20 minutes. After 20 minutes, color intensity developed was measured at 420 nm spectrophotometrically using a reaction blank containing no phosphorus.

Calibration curve of standard phosphorus was prepared from standard phosphorus solution (0.110g KH_2PO_4 per litre-0.025 mg P^{5+}/ ml) taking different concentrations (0.025, 0.05, 0.1, 0.2, 0.4 mg P); other steps being essentially similar to the one described above. With the help of standard curve, amount of phosphorus in the soil was calculated. Values are expressed as mg/g dry matter.

iii) Potassium :-

The soil sample was acid digested following the method of Toth *et.al*. (1948) in concentrated nitric acid and perchloric acid. The acid digest served as sample. Potassium is estimated according to standard flame photometric process employing ELICO Flamephtometer. Absorbance of flame color intensity was measured on Flame photometer with specific color filter for Potassium.

For standardization, various concentrations of K^+ were prepared ranging from 1-10 ppm by diluting stock solution of KCl (100 ppm). From galvanometer readings,

Potassium was estimated using calibration curve of known concentration (K^+). Values are expressed as mg/g dry matter.

iv) Calcium -

Calcium was also estimated from the sample solution flame photometrically using the same procedure of Toth *et al.*, (1948) to get acid digest. The acid digest served as sample.

For Standardization, various concentrations of Ca^+ were prepared ranging from 50-200 ppm by diluting stock solution of $CaCO_3$ (100 ppm). From galvanometer readings, calcium was estimated using calibration curve of known concentration (Ca^+). Values are expressed as mg/g dry matter.

v) Magnesium -

Magnesium was estimated following the method of Drosdoff and Nearpass (1948). To 5 ml of acid digest in a 50 ml volumetric flask, following reagents were added in a sequential order and mixed thoroughly: 1ml hydroxylamine (5% w/v), 5 ml starch compensating solution (equal volume of freshly prepared 2 % starch solution and compensating solution : 3.7 g calcium chloride, 0.6 g of trisodium phosphate all dissolved in distilled water containing 10 ml concentrated HCL and then volume made to 1 lit.), 1 ml Thiazole yellow (0.1% aqueous) and 5 ml 2.5 N NaOH. Volume was made to 50 ml with distilled water and after 30 minutes colour intensity was measured at 525 nm spectrophotometrically.

Reagent blank was prepared in the same manner as above except sample was replaced by distilled water. A standard curve for Mg^+ was also prepared with the help of different concentrations of Mg^+ from stock solution of Mg (100 ppm) and with this Mg^+ content in the sample was calculated. Values are expressed as mg/g dry matter.

vi) Sulphur -

Sulphate was estimated by adding barium chloride excess in to precipitate Barium sulphate which is then determined by turbidometry method and was measured at 420 nm.

(Tabatabai and Bremener, 1970). Soil sample 50 g each, was weighed transferred to 250 ml stopper conical flask and was shaken with exactly 50 ml of distilled water (1:1 ratio). Shaking was done by using mechanical shaker for 10 minutes. After shaking, sample was allowed to settle down for 30 minutes. The samples were filtered into Buchner funnel by using Whatman filter paper no. 42. Two ml of this filtrate is taken in a volumetric flask, diluted to 100 ml with DW and transferred into 250 ml conical flask. To this, 5.0 ml conditional reagent (by mixing 50 ml glycerol with a solution containing 30 ml conc. HCl, 300 ml distilled water, 100 ml 95% ethanol and 75g NaCl.) is added, mixing in stirring apparatus, during stirring, spoonful $BaCl_2$ crystals (about 0.2 – 0.3 g) also added. Began timing, immediately stirring for 1 min. at constant speed, immediately after stirring period has ended (at 30 sec intervals 4 min) solution was poured in silica cuvette and absorbance was measured at 420 nm by using double beam UV-VIS Spectrophotometer.

Standard calibration curve was obtained by taking volumes of 0.00 to 5.0 ml interval, 0.5 ml from the standard solution of 0.1N sodium sulphate in volumetric flask, and diluted to 100 ml distilled water. Treating standard solution with specified amount of all reagents as above procedure using distilled water as blank. Values are expressed as mg/g dry matter.

vii) Iron, Manganese, Zinc, Copper -

DTPA (diethylenetriaminepentaacetic acid) extraction method was followed (Lindsay and Norvell, 1978) by using AAS for above mentioned micronutrients analysis.

About 5 gram of soil sample was taken in extraction flask. To this, 40 ml of DTPA was added using calibrated dispenser and then sample was shaken on shaker table at 200 rpm for 2 hr. After this, samples were filtered using Whatman Filter Paper No.2 and finally filtrate was used for analysis on Atomic Absorption Spectrophotometer (Perkin –Elmer - 3030). Results are expressed as mg /gm dry matter.

d) Biological characteristics -

Soil fungi associated with *Aristolochia bracteolata* Lam. rhizosphere were isolated using standard protocol of soil serial soil dilution method on Potato Dextrose

Agar Medium and were observed daily for appearance of the fungal colonies. One gram each of soil sample was added to 10 ml DW and dilutions were made serially up to 10^{-6} level. Then each dilution extract had been spread on a petriplate containing PDA media. Pertidishes were incubated at 30^0C for 24-28 h for isolation of micro-organisms.

Composition of PDA Media (Dubey and Maheshwari – 2002)

Potato	-	200 gm
Dextrose	-	20 gm
Agar	-	17 gm
Distilled Water	-	1000 ml

e) Identification of Fungi:

For analysis of fungi grown in petriplate, samples were collected from contamination free zone and slides were prepared by using lactophenol mounting media, cotton blue as a staining medium and observed under microscope. Identification had been carried out by using standard literature (Alexopoules *et al*., 2002) as well as from the authorities of PG research centre, Department of Botany, T.C. College, Baramati.

B) Studies of *Aristolochia bracteolata* Lam.

i) Growth analysis

Healthy plants of *A. bracteolata* Lam. growing in fields with normal relative growth were subjected for further studies. Always plants from healthy populations were used for various experiments. During leaf studies, healthy, mature leaves of same stage of ontogeny were subjected for various analytical procedures.

Healthy normal plants of *A. bracteolata* Lam. at their optimal growth were collected from fields with homogenous populations. While collecting the plant sample, atmost care was taken to keep the root system intact. The collected plants were brought to the laboratory in vasculum for further growth analysis. Growth parameters like shoot and root length, number of leaves and average leaf area were recorded from at least 10 random samples. Mature plants were harvested following the same procedure and some

yield parameters like total number of pods, total seeds per pod, total seeds per plant and 100 seed weight were determined from the same.

The results obtained were subjected for statistical analysis using MINTAB software.

ii) Leaf Water Relations –

1) pH-

One gram of fresh leaves was homogenized in a mortar with pestle in 10 ml DW and from the filtrate pH was determined using Elico digital pH meter (Model Li 120) following standard calibration procedure.

2) **Moisture content** -

The moisture content was determined by weight difference method. Fully mature, intact, fresh leaves were taken and soaked in distilled water for 4 hrs, blotted to dry and then kept in oven at 80^0 C for 4 days. The difference in weight was used to calculate moisture percentage.

3) Osmotic potential (OP):

Determination of osmotic potential of cell sap from leaves was conducted as per Janardan *et al*. (1975). One gram of fresh leaf tissue was homogenized, filtered through cheese cloth and final volume was made to 25 ml by adding distilled water.

The electrical conductivity of this cell sap was measured with the help of Conductivity Bridge (Elico model No.827). Osmotic potential in bars was then calculated using formula,

$$OP = \frac{EC\ x\ 0.36\ x\ df}{0.987}$$

Where ,

EC - Electrical conductivity in mm hos cm^{-1} at 25^0C of plant extract.

Df - Dilution factor depending upon the moisture content of the tissue and extract volume.

$$\frac{Volume\ (ml) x\ Fr.wt\ (mg)}{Fr.wt + Dry\ wt.(mg)}$$

0.987= factor converting atm pressure into bars.

4) Succulence Index (SI):

Succulence index was calculated following the method of Kluge and Ting (1978),

$$SI = \frac{Fr.Wt\ (g)}{Dry.Wt.(g)}$$

(5) Mesophyll Succulence Index (MSI) -

For determination of mesophyll succulence index method of Walter (1926) was followed. Mesophyll Succulence Index was calculated using given formula –

$$MSI = \frac{Moisture\ Content\ (g)}{Total\ chlorophylls\ (mgs)}$$

iii) Germination Studies -

For germination studies, mature seeds were collected from the plants. Healthy seeds were sorted out and surface sterilized with the help of 0.1% $HgCl_2$ solution for five minutes. The seeds were then thoroughly washed with distilled water and air dried. Lots of twenty seeds each were kept in sterilized petridishes for germination over Whatman filter paper No.1. Filter paper was moistened with 15 ml distilled water. The emergence of radical from seed coat was acknowledged as criterion for germination counts which was taken after every twenty four hours. The germination was carried out in germination chamber at 28^oC under dark conditions.

Seed viability was tested by TTC (2, 3, 5-triphenyl tetrazolium chloride). If seed is viable, the 1% TTC solution obtained pink color by chemical reaction, due to respiration of the viable seeds.

To break the seed dormancy, various techniques like scarification and stratification were subjected. For this, the healthy seeds showing seed dormancy were soaked in concentrated sulphuric acid at various time intervals and, were removed, washed with distilled water and dried. The treated seeds were further allowed to germinate following standard procedure.

iv) Inorganic Constituents -

Analysis of various inorganic constituents like Nitrogen, Phosphorus, Potassium, Magnesium, Calcium, Sulphur and microelements like Iron, Manganese, Copper, Sodium and Zinc from *A. bracteolata* Lam. has been done from plant parts viz. leaf, stem and root as well as seeds. For this, mature plant was carefully uprooted, washed in water and blotted to dry. The different plant parts of *A. bracteolata* Lam. like leaf, stem and roots were carefully separated and subjected to drying separately in oven at temperature 60^{o}C. Oven dried plant material served as sample for analyzing various inorganic constituents. Methods for analysis of inorganic elements followed are as per described elsewhere in the section of soil mineral analysis. Microelements namely Iron, Manganese, Zinc, Copper were estimated on Atomic Absorption Spectrophotometer (Perkin-Elmer-3030).

The values are expressed as mg / g dry tissue.

V) Organic Solutes -

I) Total Free Amino Acids:

Total free amino acids have been estimated by method of Moore and Stein (1948). Plant sample 0.5 g of was weighed and homogenized with 5 ml of 80% ethanol. For this, homogenate was centrifuged at 15000 rpm for 15 min. The residue was re-extracted with 5 ml of 80% ethanol and centrifuged. Supernatants were collected and used for quantitative estimation of total free amino acids. 1ml of ninhydrin reagent (prepared by warming 1.25 gm of ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid with agitation, cooled and stored at 4^{0}C) was added to 0.1ml of extract in test tubes. Volume was made up to 2 ml with distilled water. Tubes were heated in a boiling water bath for 20 min. To each, 5 ml of diluent solvent (Equal volumes of water and n-propanol), was added and the contents were mixed well. After 15 min, the

absorbance of the developed purple colour was read at 570 nm spectrophotometrically. A standard curve was prepared by using L-leucine (0.1 mg/ml) . Using the standard curve, the amount of free amino acids present in the samples was calculated. Free amino acid content was expressed in terms of mg/g^{-1} fr. wt.

II) Total soluble proteins -

The total soluble proteins were estimated according to the method of Lowry *et al.* (1951). For this, 0.5 g plant material was homogenized in 10 ml 0.1 M Phosphate buffer (pH 7.0), and then filtered through Whatman filter paper No.1. Collected supernatant was kept in ice-bath and used as source for protein estimation. From the source, 0.5 ml crude supernatant was taken in test tube and diluted to 1ml with distilled water. To this, 5 ml of reagent C (Alkaline Copper Tartarate which is mixture of reagent A, 48 ml (2% Na_2CO_3 in 0.1 N NaOH) + B, 1ml (1% NaK Tartrate in H_2O) and C,1 ml (0.5% $CuSO_4$,5 H_2O in H_2O) was added. Solution was mixed well and allowed to stand for 15 minutes, at room temperature. After 15 minutes, 0.5 ml Folin Ciocalteu's reagent (prepared by dissolving 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water+ 5 ml 85% phosphoric acid + 10 ml concentrated hydrochloric acid. Refluxed for 10 hr. with addition of 15 g lithium sulfate, 5 ml water and 1 drop bromine. Refluxed for 15 min. Cooled to room temperature and brought to 100 ml with water) was added rapidly with immediate mixing. This was allowed to stand for next 30 min and the developed colour intensity was measured at 660 nm spectrophotometrically. Reagent A (2% Na_2CO_3 in 0.1 N NaOH) without source served as blank. Protein concentration was calculated by comparing with standard curve of different concentrations of BSA (Bovine Serum Albumin - 0.1 mg/ml).

Amount of proteins is expressed as mg / g^{-1} fresh wt.

III) Carbohydrates-

Total carbohydrates were determined by the phenol-sulphuric acid method (Dubois *et al.,* 1956). For this, 0.5 g plant material was hydrolysed with 10 ml 2.5 N HCl by keeping it in a boiling water bath for three hours. The content was cooled, neutralised with anhydrous sodium carbonate, final volume made 100 ml and centrifuged. Supernatant was used for estimation of total carbohydrates. For this, 0.5 ml aliquots were

taken along with different concentrations of standard glucose solution (0.1mg/ml) in other test tubes. Required amount of distilled water was added to make final volume 1 ml. For blank, instead of filtrate or standard glucose, distilled water was added to begin with the reaction. One ml of 5% phenol and 5 ml of 96% sulphuric acid was added in each test tube and all these reaction mixtures were transferred to boiling water bath for 20 minutes. After the color change occurred in tubes, tubes were taken out from bath and cooled by keeping under running tap water. The colour intensity developed was read at 490 nm using UV Spectrophotometer.

Total carbohydrates were expressed as g 100 g^{-1} fresh tissue.

vi) Enzymes -

1) Peroxidase-

The method of Maehly (1954) was followed for the assay of enzyme peroxidase. Enzyme was extracted from the mature leaves (usually 4^{th} to 8^{th} leaf) by macerating in cold distilled water in mortar and pestle. The suspension was filtered through four layered muslin cloth and filtrate was centrifuged at 10,000 rpm for 20 minutes in refrigerated centrifuge. The supernatant was used as enzyme source. Enzyme assay mixture was prepared by adding 2 ml 0.1M phosphate buffer (pH 7.0), 1 ml 20 mM Guaicol and 1ml enzyme. The reaction was initiated by addition of 0.04 ml of 10 mM H_2O_2 and the change in optical density due to oxidation of Guaicol were recorded at every 30 sec. upto 2 min. at 470 nm with frequent stirring of reaction mixture. The progressive colour change with time represented enzyme activity.

Soluble proteins were extracted from fresh tissue in 0.1 m tris HCl buffer (pH 7.5) and estimated following the method of Lowry *et al*., (1951) and calculated using standard protein curve prepared with the help of bovine serum albumin.

Enzyme activity is expressed as Δ O. D. min^{-1} mg^{-1} proteins.

2) Catalase -

For the study of enzyme catalase, a modified method of Herbert (1955) was adopted. The enzyme was extracted from mature leaves, in a manner similar to the one described for peroxidase.

The assay mixture was prepared by mixing 1ml 0.2% H_2O_2 in phosphate buffer (pH 6.8) and 1 ml aliquot of enzyme. Assay mixture was incubated at room temperature for one minute and then the reaction was terminated by the addition of 5 ml of H_2SO_4 .To this, 1ml of 10% KI solution and a drop of 2% ammonium molybdate was added. The liberated iodine was titrated against 0.01M Sodium thiosulphate using starch indicator. The difference in titration reading between two time intervals (0 and 1 min) was taken as a measure of enzyme activity.

Activity of enzyme is expressed as mg H_2O_2 broken down $min^{-1}mg^{-1}$ proteins.

3) **Polyphenol Oxidase** -

Enzyme Polyphenol oxidase was assayed as per the method of Sato and Hasegawa (1976). Leaf material 0.5 g was extracted in 10 ml 0.1M potassium phosphate buffer (pH 6.5) and filtered through two layered cheese cloth. Filtrate was centrifuged at 10,000 rpm for 10 minutes. Supernatant served as enzyme source.

A mixture of 0.2 ml enzyme sample, 0.3 ml, 0.01M Catechol, 2.5ml 0.1M potassium phosphate buffer (pH 6.5) was incubated at 25^0C. Absorbance was read at 0 and 30 sec time interval at 490 nm spectrophotometrically. The difference between two readings served as enzyme activity.

Enzyme activity is expressed as Δ O.D. $min^{-1}mg^{-1}$protein.

4) Indole Acetic acid Oxidase (IAA oxidase) -

For IAA oxidase enzyme 0.5 g leaf material was extracted in 10 ml 0.1M potassium phosphate buffer (pH 6.2) and filtered through two layered cheese cloth. Filtrate was centrifuged at 10,000 rpm for 10 minutes. Supernatant served as enzyme source.

IAA oxidase activity was determined by the method of Gorden and Weber (1951) on an aliquot of the extract by measuring residual IAA following dark incubation with constant shaking at 30^0 C. The enzyme incubation mixture contained 2 ml 0.1M phosphate buffer (pH 6.2), 1ml 0.006 M Para Coumaric acid (PCA), 4 ml 5.7 mM IAA, 1ml 30 µM $MnCl_2$, and 2.0 ml enzyme preparation giving a total volume of 10 ml.

Reaction was initiated by addition of IAA to RM. Residual IAA was determined spectrophotometrically according to Gordon and Weber's modification of the Salkowski test, except that Fe $(NO_3)_3$ was replaced by $FeCl_3$. Aliquots of 2.0 ml were withdrawn from the incubation mixtures after 0 and 30 min incubation and placed in flasks containing 5.2 ml of 5 M Perchloric acid ($HClO_4$),0.5 ml of 0.1M $Fe(NO_3)_3$, and then diluted to 10 ml with distilled H_2O. After incubating the Salkowski mixtures in the dark for 30 min, Absorbance was read at 535 nm spectrophotometrically and the difference between two readings served as enzyme activity.

Enzyme activity is expressed as Δ O.D. $min^{-1}mg^{-1}$protein.

5) Nitrate reductase (NR) -

The activity of *in vivo* nitrate reductase was assayed according to the method described by Evans (1982). This enzyme was assayed from leaf tissue. For this, 0.5 g freshly harvested leaf tissue, cut into small pieces, was suspended in 5 ml mixture of 0.1 M phosphate buffer (pH 7.0), 0.1 M KNO_3, 5% n- propanol, and 2 drops of chloramphenicol (0.5 mg/ml) in sealed jars and was incubated at 25^0C in the dark for 60 min. After 60 min nitrate reductase was measured by determining NO_2 production, which was estimated by treating 0.4 ml of incubation mixture with 0.3 ml each of 1% sulphanilamide and 0.02% NEEDA (N-1- Naphthyl- ethylenedimine hydrochloride) for 20 minutes. The samples were diluted to 2.2 ml distilled water. The absorbance was measured at 540 nm spectrophotometrically. Standard curve was prepared following different concentrations of $NaNO_2$ (10, 20, 30, 40 µg) by using same procedure of NO_2 estimation.

The enzyme activity is expressed as µg NO_2 consumed $h^{-1}g^{-1}$ fresh tissue.

6) Nitrite reductase (NiR)-

Enzyme nitrite reductase from leaf tissue was assayed following the method of Rama Rao *et al., (*1983) with slight modifications. Here, 0.5 g freshly cut leaf tissue was incubated in 5 ml reaction mixture containing 0.02 M KNO_2, 5% n-propanol and 0.05% Triton –X-100 in light for 60 minutes. The enzyme activity was detected by

determining consumed NO_2 than in 0 min. reaction using sulphanilamide and NEEDA with a small aliquot of RM.

Enzyme activity is expressed as µg NO_2 liberated $h^{-1}g^{-1}$ fresh tissue.

vii) Photosynthesis –

a) Chlorophylls -

Total Chlorophylls were estimated according to the method of Arnon (1949). Chlorophylls were extracted in chilled 80% acetone from 0.5 g leaf material in a cold room. The extract was filtered through Buchner funnel using Whatman No.1 filter paper. Residue was washed repeatedly with 80% acetone. Collecting the washings in the same filtrate, the volume of the filtrate was made 100 ml with 80% acetone. The absorbance was read at 645 and 663 nm wavelength spectrophotometrically using Elico spectrophotometer 24 C.

Chlorophylls (mg 100 g^{-1} fresh tissue) were calculated using the following formulae–

Chlorophyll a $= 12.7 \times A_{663} - 2.69 \times A_{645} = X$

Chlorophyll b $= 22.9 \times A_{645} - 4.68 \times A_{663} = Y$

Chlorophyll a+b $= (A_{663} \times 22.2) + (A_{645} \times 8.02) = Z$

Chlorophyll a / b / a+ b

$$\text{mg } 100\ g^{-1} \text{ fresh tissue} = \frac{X / Y / Z \times \text{Vol. of extract (ml)} \times 100}{1000 \times \text{Wt. of plant material (g)}}$$

b) Carotenoids -

Carotenoids were estimated by reading the absorbance of above acetone extract at 480 nm (Kirk and Allen, 1965). Total carotenoids were estimated using formula of Liaaen-Jensen and Jensen (1971).Values of carotenoides are expressed as mgs $100g^{-1}$ fr. tissue.

$$\text{Carotenoids} = A_{480} + 0.114 \times A_{663} - 0.636 \times A_{645}$$

c) Chlorophyll Stability Index (CSI) and Carotenoid Stability Index (CaSI)

Chlorophyll Stability Index was determined following the method of Kaloyereas (1958). Fresh leaf material (0.5 g) was taken, washed, blotted to dry, cut into small pieces and then boiled in 50 ml distilled water for 30 minutes at 60^0C. After boiling, the leaves were subjected to extraction with 80 % acetone. Another control set was prepared without boiling treatment.

Chlorophyll a, Chlorophyll b and total chlorophylls and carotenoids were estimated from the extract by the method of Arnon (1949) and Kirk and Allen (1965) respectively for treated and untreated leaves as described earlier. The difference between two readings was used to calculate the Chlorophyll Stability Index and carotenoid stability index, respectively.

d) Photo-oxidation of Chlorophylls:

Chlorophylls were extracted in chilled 80% acetone from 0.5 g leaf material in a cold room. The extract was filtered through Buchner funnel using Whatman No.1 filter paper. Residue was washed repeatedly with 80% acetone. Collecting the washings in the same filtrate, the volume of the filtrate was made 100 ml with 80% acetone. The absorbance was read at 645 and 663 nm wavelength spectrophotometrically using Elico spectrophotometer 24 C. For study of photooxidation chlorophylls extracted in acetone kept for different treatment like heat i.e extract heated at 40^0 C, some amount of extract is kept in dark period for 24 hrs and for light treatment extract is kept in natural light for 24 hrs. After 24 hrs. by all the treatments total Chlorophylls were estimated according to the method of Arnon (1949).

e) Hill Reaction -

The Hill reaction was determined following the method of Witham *et al.* (1971). For this, 10 g of mature leaves were homogenized in 20 ml prechilled 0.1 M phosphate buffer (pH 6.5) containing 0.3 M sucrose. The homogenate was centrifuged and residue was washed several times with buffer and at the end, the residue was resuspended in 5 ml phosphate buffer. The Hill reaction was initiated

with 1 ml of DCPIP (2, 6 Dichlorophenol Indophenol) in phosphate buffer and 1 ml chlorophyll suspension. The oxidation of DCPIP was determined by loss in colour, which was measured at 620 nm spectrophotometrically, by taking readings at every one min. time interval.

The activity is expressed as Δ O.D. min^{-1} mg^{-1} chl.

viii) CAM Studies in *Aristolochia bracteolata* Lam.

I) Titratable Acidity Number (TAN) -

Diurnal fluctuations in titratable acidity number (TAN) was determined by the method of Thomas and Beevers (1949) from fresh leaves. One gram of plant material was boiled in 60 ml of distilled water for 30 min. and final volume made 100 ml D.W. and filtered. The known volume of filtrate was titrated against N /40 NaOH using phenolphthalein as an indicator. NaOH was standardized against standard N/40 oxalic acid. TAN values were calculated using following formula -

$$TAN = \frac{\text{Vol. of oxalic acid (ml)}}{\text{Titration reading (ml)}} \times \frac{\text{Total vol. of extract (ml)}}{\text{Wt. of plt. Mate. (g)}} \times \frac{\text{Extract reading (ml)}}{\text{Vol. of extract taken For titration (ml)}} \times \frac{100}{4}$$

II) pH -

1 gram of fresh leaf was homogenized in 25 ml distilled water and filtered through double layered muslin cloth. Filtrate is used for measurement for pH on digital pen pH meter.

TAN and pH studies of leaf cell sap were calculated at every 3 hr interval in whole day cycle from fresh samples. These studies were conducted continuously for 72 hr at summer (Apr.- May.), rainy season (July – Aug.) and winter (Nov. – Dec.) in a year 2010-11 and 2011-12 respectively.

III) Stomatal Studies -

Morphology of mature leaf stomata was studied by preparing slides of thin peelings of lower and upper epidermis mounted in glycerine and by using camera lucida technique. Sketches were made for further studies. Stomatal behaviour was checked after every three hours throughout the day and night, and observations were recorded, respectively.

ix) Secondary metabolites -

a) Free Proline -

Free proline content was determined according to the method of Bates *et.al.;* (1973). For this, 0.5 gm of fresh plant material was homogenised in 10 ml sulphoslicylic acid (3.0%) and then filtered through Whatman filter paper No.1. Two ml of this filtrate was reacted with 2 ml glacial acetic acid and 2 ml acid Ninhydrin reagent (1.25 gm of Ninhydrin in a mixture of 30 ml of glacial acetic acid and 20 ml of 6 M orthophosphoric acid) in a test tube for one hour at 100^0 C in boiling water bath. Similar procedure was followed using Standard proline solution (1 µg / ml proline). After boiling, the reaction was terminated by transferring the test tubes immediately to ice bath. The color developed in reaction mixture was extracted using 4 ml toluene. The RM was mixed vigorously with test tube stirrer for 15-20 sec. and allowed to settle. Reaction mixture was then brought to room temperature and absorbance of toluene chromophore was measured at 520 nm spectrophotometrically using toluene blank. Free Proline content was calculated from calibration curve and the values of free proline were expressed as µg 100 g^{-1} fresh tissue.

b) Total Polyphenols -

Total polyphenols were determined according to Folin and Denis (1915). Fresh leaf material (0.5 g) was homogenized in 30 ml 80 % acetone and filtered through Buchner Funnel. The residue was washed several times with 80 % acetone and the final volume of the filtrate was made 100 ml with 80 % acetone. Two ml extract along with series of standards (Standard Tannic acid 0.1 mg/ml) was taken in Nessler's tube, to this 10 ml 20 % Na_2CO_3 and 2 ml Folin Denis Reagent (750 ml of distilled water, 100 g sodium tungstate, 20 g phosphomolybdic acid and 50 ml ortho phosphoric acid, mixture

was refluxed for 2 hrs, cooled and diluted to 1 litre) were added. The final volume was made 50 ml with distilled water. After 20 min. absorbance of developed color was read at 660 nm spectrophotometrically using reagent blank. Total polyphenols were calculated using standard curve of Tannic acid and are expressed as g 100 g^{-1} fresh tissue.

c) Tannins –

The tannin content in dried plant parts was estimated spectrophotometrically by Folin-Denis method (Schanderl, 1970).The method is based on the oxidation of molecules containing a –OH groups. For this, 0.5 g plant material was hydrolysed with 70 ml DW in 250 ml volumetric flask by keeping it in a boiling water bath for 30 minutes. The content was cooled, centrifuged at 10,000 rpm for 20 minutes and supernatant was collected in 100 ml volumetric flask. Final volume made 100 ml using distilled water and this served as source of tannins. One ml of the sample extract, 5 ml of Folin-Denis reagent (750 ml of distilled water, 100 g sodium tungstate, 20 g phosphomolybdic acid and 50 ml ortho phosphoric acid, this mixture was refluxed for 2 hrs, cooled and diluted to 1 litre). 10 ml of 20% Sodium carbonate was added and volume was made 100 ml with distilled water. The absorbance was measured spectrophotometrically at 700 nm within 30 minute of reaction against the blank prepared in similar manner without tannic acid. The intensity of tannins in the solution was estimated against standard tannic acid.

The total tannin content, expressed as mg tannic acid 100 g^{-1} dry weight.

C) Aristolochic acid analysis –

1) Preparation of plant material –

Mature root, stem and leaves of *Aristolochia bracteolata* Lam., were collected from the agricultural and open fields around Baramati. Collected material was washed thoroughly in running tap water, rinsed in distilled water, shade dried in open air and ground to powder.

2) Quantification of Aristolochic acid with respect to extraction in different extracts –

Methanolic and ethyl acetate extracts were prepared by taking about two grams of oven dried powder of root, stem and leaf of *Aristolochia bracteolata* Lam. All the extracts were centrifuged at 10000 rpm for 10 minutes. Supernatants were collected and filtered through syringe filter (PVDF 0.22µ), stored in HPLC vials and used for further HPLC analysis.

Samples from each solvent extracts of root, stem and leaf were injected on HPLC system (Shimadzu, LC solution 2010) equipped with UV detector. HPLC system maintained with the flow rate 1ml / min and UV detector wavelength 390 nm. Column used was C18, 250 mm x 4.6 mm, 5µm. Extracted samples of methanol and ethyl acetate were injected (20 µl) in HPLC system separately and corresponding chromatograms were recorded. The graphs obtained were subjected for further confirmation of components detected in samples.

3) Isolation of Aristolochic acid -

Dry powder (25 gm) of *Aristolochia bracteolata* Lam. was dissolved in methanol at room temperature and kept overnight. Then undissolved material was removed by filtration method. This methanolic extract filtrate was further treated with saturated $NaHCO_3$ solution for transfer of acid into its sodium salt. Then, aqueous solution of sodium salt of Aristolochic acid acidified with concentrated HCL to get isolated carboxylic acid in solid form. This solid form is further dried under IR lamp to get the dark brown colored solid residue of aristolochic acid.

4) Characterization of Aristolochic acid –

Isolated acid extract was analyzed for identification by different analytical techniques viz. Thin Layer Chromatography (TLC), Gas Chromatography - Mass Spectroscopy (GC-MS), (Fourier Transform Infrared (FT- IR) Spectroscopy, Proton Nuclear Magnetic Resonance spectroscopy (^{1}H-NMR), High Pressure Liquid Chromatography (HPLC), X-ray Crystallography (XRD).

a) TLC analysis –

Methanolic extract of isolated aristolochic acid from *Aristolochia bracteolata* Lam. were separated on TLC aluminum sheets aluminium sheets 20 x 20 cm,1 mm thick, silica –gel 60 F_{254} (Merk Germany) in the mobile phase- n hexane: ethyl acetate (80:20). After completion of the solvent run, plates were examined under ultraviolet light (254 and 365 nm; Uvitec-LF-206.LS, UK). Single spot was obtained, which was located as a transleucent spot under UV light.

b) GC-MS analysis –

Methanolic extracted solid sample of isolated Aristolochic acid was used for GC-MS analysis. GCMS model Shimadzu QP 2010 ultra equipped with electronic impact ionization (EI) mode of having energy 70eV, High vacuum system, wide mass range, direct injection unit (DI) for sample injection. Helium was used as the carrier gas. Analysis parameters were,

;ource temperature		^{0}C
·face temperature		C
·ent cut time		nin.
Start time	:	0.01 min
End time	:	20 min,
Acquisition mode	:	Scan
m/z start	:	80 amu
m/z end	:	800 amu.
Mass temperature programmed	:	350 ^{0} C at the rate of 20^0 C/ min, held for 2 minutes.

Isolated sample was injected through DI mode. Mass spectrum was recorded. Quantitative determination was made by relative intensities of mass spectra.

c) FT-IR analysis -

The spectrum of isolated acid is often best determined by alkali-halide pellet preparation because alkali halide does not show interference in the IR region 4000-650 cm^{-1}. About 200 mg of alkali halide (KBr) was ground together and pressed at room temperature under high pressure into small disc to form uniform pellet, scanned on Nicolet FT-IR spectrometer in the IR region 4000-650 cm^{-1}, obtained spectrum for blank correction. About 2 mg of isolated acid and 200 mg of dried alkali halide (KBr) were ground together and pressed at room temperature under high pressure into small disc to form uniform pellet. A isolated acid pellet scanned on Nicolet FT-IR spectrometer in the IR region 4000-650 cm^{-1}, obtained complete spectrum, showing the characteristics absorption bands are recorded in wave number in the region spectrometer in the region of 4000 to 650 cm^{-1} (function of wavelength).

d) ^{1}H-NMR analysis –

The ^{1}H-NMR spectra were recorded at 400 MHz using Bruker spectrometer in DMSO – d^{6}. About 1 mg of sample dissolved in 1 ml of DMSO-d^{6} in sample tube and rotated during spectral determination to minimize any magnetic anisotropy. Spectrum is scanned by field sweep method (Kalsi, 2002). Where magnetic field is varied. Thus, when nucleus absorbs energy, resonance occurs which causes a tiny electrical current to flow in a coil surrounding the sample tube. Amplified current is obtained on single chart paper.

e) HPLC analysis –

The separation and quantification of aristolochic acid was achieved on HPLC system (Shimadzu, LC solution 2010) equipped with UV detector. HPLC system maintained with the flow rate 1ml/ min and UV detector wavelength 390 nm. Column used was C18, 250mm x 4.6 mm, 5µm. Mobile phase was prepared in composition of Methanol : Water : Acetic acid (67:32:1). Isolated

acid sample was prepared in mobile phase and injected 20 µl in HPLC system, recorded corresponding chromatogram.

f) X-ray crystallography analysis –

The methods of X-ray diffraction to identify the crystal structure and determining the space group of the crystal are well documented. (Kluge and Alexander, 1974). It is used primarily to examine the crystalline structure of materials and to determine bond length and angles (Pomeraz and Meloan, 1971). Structural properties of aristolochic acid were studied by analyzing the X-ray diffraction patterns obtained using Philips X-ray diffractometer Model PW 3710 for λ = 1.05406 and 2.2897 A^0 for Cu- K_α and Cr-K_α radiation respectively. The 2Θ, d value and intensity were compared from the available literature. The measured intensity as a function of the angle is compared with A standard d values using computer software (JCPDS – International centre for diffraction data, USA (1977) on X ray diffraction, which completes the identification of the crystal structure.

D) Effect of Water Stress -

I) Water Stress Studies:

Seeds of *Aristolochia bracteolata* Lam. were grown in earthen pots with a soil, sand, vermin-compost mixture and watered daily. Well grown plants were subjected treatment of water stress. To study the effect of water stress on these mature plants, following four treatments were subjected, namely -

Control = Control plants (daily watered)
24 hrs = Water deficit for one day
48 hrs = Water deficit for two days
72 hrs = Water deficit for three days

Treated plants were subjected for analysis of effect of water stress. For this, plants were uprooted and roots were washed carefully with water to clear away the adhered soil particles. Intact plants were brought to the laboratory. Healthy and mature plant parts were separated carefully and separated plant parts like leaf, stem and roots

were subjected for analysis. Also some plant material was oven dried following usual oven drying procedure and dried powdered plant material was used for analysis as per requirement. The plant analysis with respect to various biochemical aspects like growth analysis, mineral nutrition, organic constituents, enzyme systems, photosynthesis, secondary metabolites from fresh or oven dried plant material (wherever necessary) was performed according to the methods described earlier.

E) Antimicrobial Studies -

i) Antibacterial Studies -

a) Plant Material

Mature root, stem and leaves of *A. bracteolata* Lam., were collected from the agricultural as well as open fields from collection sites around Baramati. Collected plants are washed thoroughly in running tap water, rinsed in distilled water, shade dried in open air and ground to powder. The powdered plant material was subjected for further analysis.

b) Preparation of Extracts

Five gram of plant material was dissolved in 50 ml of solvent (Aqueous, Petroleum ether, Butanol, Methanol) and centrifuged for 10 min. at 10,000 rpm. Obtained supernatants were filtered with Whatman Filter paper No.1 and evaporated to dryness.Dried residue was dissolved in minimal volume of Dimethyl Sulphoxide and stored at 4^0C until used in the assay.

c) Test Organisms -

Bacterial cultures were obtained from Post Graduate Department of Microbiology, Tuljaram Chaturchand College, Baramati. One strain of Gram positive bacteria- *Staphylococcus aureus* and two strains of Gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa* were used as target organisms to evaluate antibacterial activity. All these bacteria were isolated from clinical samples like urine and pus by streak plate method on Nutrient Agar, identified by cultural and microscopic observations and biochemical testing as per Bergy's Manual and Determinative

Bacteriology. All bacterial cultures were maintained on nutrient agar slants at 4^0C and periodically sub cultured.

d) Antibacterial Assay -

In vitro antibacterial activity of the different extracts of *Aristolochia bracteolata* Lam. was studied by disc diffusion method (Ronald *et.al.;* 1989) against different bacterial strains such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Plant extracts from different solvents (0.1 ml) each was loaded on a sterile filter paper disc 6 mm dia. and dried aseptically. The solvent (0.1 ml) loaded without extracts served as control. A standard disc of ampicillin (50µg) was used as control. Overnight grown bacterial culture (0.1ml) was diluted into 10 ml saline solution and poured on sterile nutrient agar.

These inoculated plates were kept in refrigerator for half an hour for prediffusion and finally incubated at 37±1° in incubator for 24 to 48 hours to allow growth of organisms. After incubation, developed colour inhibition zone diameter around the disc was recorded on plates in which inhibition of target organism was noted. Clear zone of inhibition around disc showed the presence of antibacterial activity.

ii) Antifungal Studies -

a) Plant Material-

Mature root, stem and leaves of *Aristolochia bracteolata* Lam., were collected from the agricultural and open fields around Baramati. Collected material was washed thoroughly in running tap water, rinsed in distilled water, shade dried in open air and ground to powder.

b) Preparation of Extracts-

Five grams of dried and powdered plant material was dissolved in 50 ml of solvent (Aqueous, Petroleum ether, Butanol, Methanol) and centrifuged at 10,000 rpm for 10 minutes. Obtained supernatants were filtered with Whatman Filter paper No.41. Supernatant was evaporated to dryness at 60^0C, the extract was then weighed, dissolved

in minimal volume of Dimethyl Sulphoxide (DMSO) and stored at 4^0C until used in the assay.

c) Test Organisms-

Fungi used in this study were chosen primarily on the basis of their importance as opportunistic pathogens of humans. The following fungi were used for experiments, *Aspergillus whentii, Fusarium monoliformae* and *Penicillum imblicatum*. Fungal cultures were obtained from National Chemical Laboratory, Pune. All fungal cultures were maintained on Potato Dextrose Agar slants, stored at 4^0C and periodically subcultured.

d) Antifungal Assay -

In vitro antifungal activity in the different extracts of *Aristolochia bracteolata* Lam. was studied by disc diffusion method (Ronald *et.al.*; 1989) using different fungal strains such as *Aspergillus whentii, Fusarium monoliformae* and *Penicillum imblicatum* as target fungi. These extracts were loaded separately (0.1ml) on a sterile Whatman filter paper No. 41 filter paper disc with 6 mm dia. and dried aseptically. The solvent

loaded without extracts were served as negative control. A standard disc of Nystatin (50µg) was used as positive control. Four day old fungal suspension (0.1ml) was poured on sterile Petri dish of potato dextrose agar. After 4 h, the extract and control loaded discs were placed in refrigerator half an hour for prediffusion, and finally incubated at 37±1° in incubator for 24 to 48 hr to allow growth of fungal culture. After incubation, the growth and zone of inhibition in plates was observed; the diameter of the zone of inhibition was measured and recorded. Clear zone of inhibition around the disc indicated the presence of antifungal activity.

All experiments were replicated three times and values presented are means of three replications.

F) Statistical analysis –

The data obtained in various experiments was subjected to statistical analysis. For this, expertise was obtained from Post Graduate department of Statistics, Tuljaram Chaturchand College. Baramati. Also the primary data was analyzed using MINITAB software and the values presented in tables and figures are statistically processed wherever necessary.

CHAPTER IV

RESULTS AND DISCUSSION

A) Habitat Analysis:

a) Study area-

Baramati (18^0, 8' N and 75^0, 7' E) is a taluka place of Pune district in Maharashtra, India. This region is a distinct semi-arid zone because of the rain shadow effect of Western Ghats (Plate 1). This place has mean annual maximum temperature 33.47^0C and minimum 20.03 ± 2.00^0C (Murumkar, 1993). Highest daily temperature is recorded during May (mean max.36.95^0C) and lowest in December (mean max.17.07^0C). During summer, especially in April and May, daily maximum temperature many a times shoots up beyond 40^0C. On an average this region is dry with almost 7 months of dry hot period.

The relative humidity is highest during months of September (83.69%) while the lowest recorded in April (55.11%).The rate of evapotranspiration is maximum in May (11.34 mm/h) while lowest in December (3.24 mm/h).

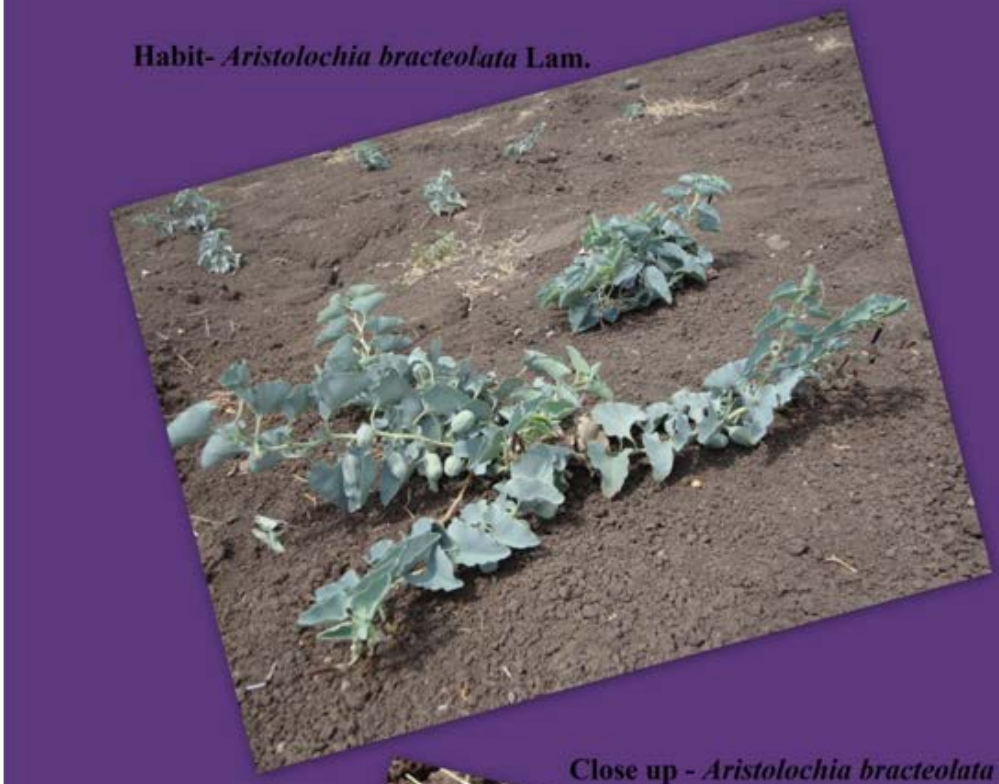
From edaphic point of view, a wide range of soils occur in this region. Right from laterite to red soil and to black cotton soils occur in this area. The red and black cotton soils are sodic in nature and these show overall deficiency of potassium. The water holding capacity also varies from 30.26% to 76.18% (from unpublished data). The soils under irrigation, especially black cotton soils are rich in humus.

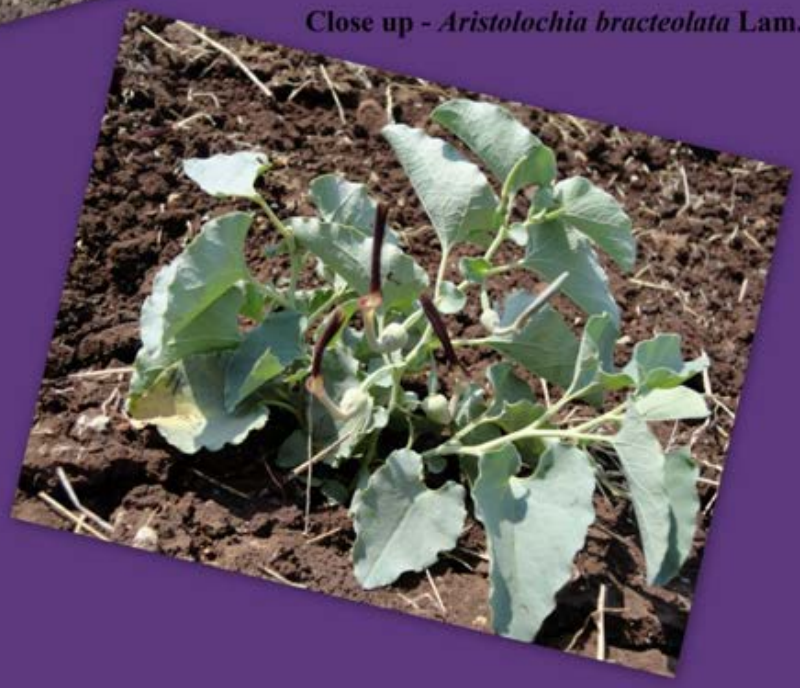
Taxonomic survey of this region showed that some weed populations dominate this area either in cropping season i.e. "in season" or after cropping season i.e. "off the season" (Murumkar and Magdum, 1991) or throughout the year. After preliminary survey of weed populations of this region, *Aristolochia bracteolata* Lam. is selected as an experimental material which is a dominant weed of this region.

b) Life Form -

The mature, healthy flowering – fruiting plant of *Aristolochia bracteolata* Lam. is studied for its morphological diagnosis. This taxonomic analysis is in tune with the taxonomic survey of available records like Cooke

Plate 1: Habitat of *Aristolochia bracteolata* Lam.





(1901-1908), Wealth of India (1982), Flora of Maharashtra by Deshpande (1993). The taxonomic details are as -

The plant is a slender, prostrate, perennial. Stems are herbaceous 30-45 cm long weak prostate, branched, straight, glabrous leaves are 3.8-7.5 cm long and as broad as long, reniform or broadly ovate, usually obtuse cordate at the base with glaucous beneath, finally reticulately veined, glabrous petiolate 1.3 - 3.2 cm long. Flower is solitary axilary, dark purple, perianth tube 4-5cm long, the tube inflated below, then contracted into a cylindrical neck, and expanded in a dialated oblique limb with revolute margins. Pedicles are with large sessile orbicular or subreniform bract at the base. Stamens are 6, adnate and around the style column. Ovary is inferior 6 celled, ovules many, 2 seriate. Fruit is a septicidal capsule, 12 ribbed, splitting through placentas. Perianth 2.5-4.5 cm long, base subglobose type, cylindrical with a trumpet shaped mouth, lip linear, dark purple, finely reticulately veined as long as tube, capsule 1.3 – 2.0 cm long, oblong. Fruit is ellipsoid 12 ribbed capsule, glabrous. Seeds are 6 mm long, deltoid, with slightly cordate base, endospermic. Flowering period October to May. Every part of the plant is nauseously bitter and taste remains for a long time chiefly in the throat.

In present case the success of weed *Aristolochia bracteolata* Lam. lies in successful seasonal germination pattern, perennial root stalk, physiological capacity to drought tolerance, adapted life form and efficient dispersal of their fruits and seeds.

c) Rhizosphere Soil analysis :

Soil is a natural surface layer capable of supporting plants. Soil forms the upper-most layer of earth's crust and is made up of inorganic and organic matter. Important plant soil nutrients include nitrogen (helps leaves and stem grow), phosphate (helps root and fruit develop) and potassium (stimulates overall plant health). When plant dies, it returns the nutrients, as initially observed, from the soil back to the soil and enrich the soil. In this way soil plays a very important role in recycling of nutrients (Jenking, 2008).Soil is comprised of minerals, organic matter, water and air. The composition and proportion of these components greatly influence the physical properties of soil including its composition, texture, structure, and porosity. In turn, these properties

influence the movement of air and water in the soil, and thus help the soil to function better to aid plant growth.

a)Physical Properties: -

1) pH :

The values of pH of soil associated with open field and cultivated field of *Aristolochia bracteolata* Lam. are recorded in Table 1. The pH of soil associated with *Aristolochia bracteolata* Lam. at open field was 8.16 and at cultivated soil 8. 38 which falls in medium alkaline range.

Soil pH refers to a soil's acidity or alkalinity and is the measure of hydrogen ions (H^+) in the soil. The soil pH and its interpretation is shown in Table 2 (Winston, 1968).

A high amount of H^+ corresponds to a low pH value and vice versa. The pH scale ranges from approximately 0 to 14 with 7 being neutral, below 7 acidic, and above 7 alkaline (basic). Soil pH value depends upon number of factors like soil moisture, dissolved CO_2 , composition of exchangeable cations, nature of cation exchange materials, composition and concentration of soluble salts, presence or absence of gypsum, type of vegetation and presence of alkaline earth carbonates. Soil pH can affect cation exchange capacity (CEC) and Anion exchange capacity (AEC) by altering the surface charge of colloids. A higher concentration of H^+ (lower pH) will neutralize the negative charge on colloids, thereby decreasing CEC and increasing AEC. The opposite occurs when pH increases. Soil pH influences the solubility of nutrients. It also affects the activity of microorganisms responsible for breaking down organic matter and most chemical transformations in the soil. Soil pH thus affects the availability of several plant nutrients.

A pH range of 6 to 7 is generally most favorable for plant growth because most plant nutrients are readily available in this range. However, some plants have soil pH requirements above or below this range. Mali (2012) studied salt affected soils from Baramati tahsil and observed that pH of soils fluctuated between 7.90- 8.29. Joshi in

Table 1- Physical characters of soil associated with *Aristolochia bracteolata* Lam. from open and cultivated fields.

Parameter	Open Field	Cultivated Field
pH	8.16 ± 0.03	8.38 ± 0.02
EC (dSm^{-1})	0.22	0.35
$CaCO_3$ (%)	14	8
Organic carbon (%)	0.86	0.10

Data represents average of three determinations

Table 2 : Soil pH and its Interpretation (Winston, 1968).

Range	Denomination
> 4.0	Very strongly acidic
4.0 > 5.5	Strongly acidic
5.5 > 6.0	Medium acidic
6.0 > 6.5	Slightly acidic
6.5 > 7.0	Very slightly acidic
7.0 > 7.5	Very slightly alkaline
7.5 > 8.0	Slightly alkaline
8.0 > 8.5	Medium alkaline
8.5 > 10.0	Strongly alkaline
>10	Very strongly alkaline

1982 also observed that pH of saline soils supporting succulent halophytes was between 7.5 to 8.2.

From the obtained results it is observed that *Aristolochia bracteolata* Lam. needs alkaline soil for its normal growth even at different fields.

2) Electrical conductivity (EC):-

EC associated with open and cultivated field of *Aristolochia bracteolata* Lam. is recorded in Table 1. It was found that EC was 0.22 dSm^{-1} at open fields and at cultivated field 0.35 dS m^{-1}.

From the soil EC soluble salts present in a soil can be estimated. It can be defined as the "Reciprocal of electrical resistance and expressed as reciprocal of ohms or simens. EC is independent of the size and shape of soil sample. EC measurements can be made quickly and accurately and hence used for estimating soluble salts in soil (Richards, 1954).

EC of soil indicates the cation exchange capacity of soil. In normal soils, Ca and Mg are principally absorbed on soil particles. The soil properties like dispersion of particles, infiltration, permeability, structure, stability of aggregate etc. are sensitive to exchangeable cations, mainly Ca. If EC of saturated soil solution is 4 dS m^{-1} or more then the soil is considered as saline.

EC values are indicative of free ion availability and hence unweathered sediments would be expected to have lower values. Weathered sediments with a higher availability of free ions would be expected to have an EC of 2-4 dS m^{-1}. The sediments have only low EC values ranging between 0.2 to 0.5 dS m^{-1}, suggesting that the availability of free ions in the sediments is extremely limited (Brady, 2002).

Our results also fall within above mentioned range confirming studied soils are alkaline in nature. Also, low EC values indicate less free ion availability and poor soil condition. Thus there is strain on plants to avail essential nutrients from such soil.

3) Calcium carbonate ($CaCO_3$) :

Amount of $CaCO_3$ in open soil and cultivated field of *Aristolochia bracteolata* Lam. are recorded in Table 1. The soil $CaCO_3$ content of the studied locations were 14 % and 8 % from open and cultivated fields, respectively.

Chemically, limestone is calcium carbonate ($CaCO_3$), and as this compound dissolves, the soil pH decreases or becomes more acidic. Limestone is responsible for alkaline soil pH (7.0-8.5). A product of this reaction is free calcium and carbon dioxide, which is given off as a gas. Soils can contain up to 60% limestone. In other words, an acre furrow slice of soil (2,000,000 pounds) can contain up to 1,200,000 pounds of limestone. However, most arid soils contain around 2 to 10% limestone (40,000 to 200,000 pounds).

pH of soils is mainly controlled by the amount of calcium carbonate in the soil profile and often fluctuating between 7.5 to 8.2. Existence or absence of calcium carbonate has an important effect on soil pH and therefore, controlling many chemical reactions in relation to nutrient availability for plants and mobility of these elements in soil (Sarmadian, 2010). Soil survey staff (1999) indicated increase in soil pH with increasing soil $CaCO_3$ content. Mali (2012) observed $CaCO_3$ content varied from 6.89 to 20.21% at Kambleshwar location in study of salt affected soils from Baramati region.

In our findings also as open soil was slightly alkaline with pH 7.79 had 14% $CaCO_3$ while agricultural soil having pH 7.68 was with 8% $CaCO_3$. These results are in accordance with soil survey staff (1999) findings as there is positive relation in pH and $CaCO_3$ content and these further reflect on their influence of availability of free cations and anions in the soil thereby limiting soil fertility.

4. Organic Carbon:

Amount of organic carbon in open and cultivated field of soil associated with *Aristolochia bracteolata* Lam. is recorded in Table 1. The organic carbon content of the studied locations was 0.86 % and 0.10 % from open and cultivated fields respectively.

Soil is the largest pool of terrestrial organic carbon in the biosphere, storing more C than is contained in plants and the atmosphere combined (Schlesinger, 1977). The abundance of organic C in the soil affects and is affected by plant production, and its role as a key control of soil fertility and agricultural production has been recognized for more than a century (Tiessen *et al.*,1994).

Total organic carbon (TOC) is the carbon (C) stored in soil organic matter (SOM). Organic carbon (OC) enters the soil through the decomposition of plant and animal residues, root exudates, living and dead microorganisms, and soil biota. SOM is the

organic fraction of soil exclusive of non decomposed plant and animal residues. Soil Organic Carbon (SOC) is the main source of energy for soil microorganisms. SOC is one of the most important constituents of the soil due to its capacity to affect plant growth as both a source of energy and a trigger for nutrient availability through mineralization. SOC fractions in the active pool, previously described, are the main source of energy and nutrients for soil microorganisms. Humus participates in aggregate stability and nutrient and water holding capacity. An increase in SOM, and therefore total C, leads to greater biological diversity in the soil, thus increasing biological control of plant diseases and pests.

Soil organic carbon is the main chemical indicator of soil quality (Nortcliff, 2002). Arid and semi-arid region cover at least 30% of the global continental area and contains 20 % of the global soil carbon stocks (Bechtold and Inouye, 2007). Semi-arid and arid regions are important source and sinks of carbon (Stone, 2008). Zhang and his coworkers (2013) recently recorded that organic carbon can accumulate with increase of plantation age.

According to White *et al.* (2009) semi-arid and arid regions, which are particularly sensitive to global change, account for about 40% of the terrestrial surface of Earth and contain approximately 10% of the global soil organic carbon stock.

Austin (2011), states that there is no consistent conclusion on net carbon effect of semi-arid and arid regions on carbon sequestration partially because of the unique characteristics of arid ecosystems like exceptional vulnerability and spatial heterogeneity. Also complex responses of biogeochemical cycling to a range of environmental conditions, such as water availability, precipitation pulse, solar radiation, or even heat and wind.

Jobbagy and Jackson (2000) gave the vertical distribution of soil organic carbon upto 64% for 0-100 cm, 21% for the depth layer 100-200 cm and 15% for the layer 200-300 cm. Globally, the concentration of the amount of soil organic carbon in the layer 0-20 cm ranged from 29% in cold and arid regions under shrub land to 57% for cold and humid forests.

Therefore, our results suggested that the open fields associated with *Aristolochia bracteolata* Lam. may accumulate more soil organic carbon than cultivted fields because

of large availabilty of organic matter in open fields than cultivated fields. Perhaps this may reflect in growth of fungal colonies.

b) Chemical Characters

1. **Nitrogen:**

Nitrogen content of soil in open and cultivated field of *Aristolochia bracteolata* Lam. is recorded in Table 3. It is higher in open field (8.14 mg / gm dry matter) and lower in cultivated field (5.57 mg/gm dry matter).

Nearly all organic nitrogen present in soil originates from biological life (Loll and Bollag, 1983). Most of the soil nitrogen is very stable and resistant to biological decomposition.

In arable soil, amino N can make up about 30% of the total nitrogen present and may amount to 1700-2000 kg N/ ha in the upper layer (10 cm) (Catroux and Schnitzer,1987). Total soil N content ranges from < 0.02 % in subsoils to > 2.5 % in organic soils. The N occurs as inorganic form (ammonium, nitrite, nitrate, nitrous oxide, nitric oxide) or organic form (proteins amino acids amino sugars and other complex N compounds), where about 95% of total N in surface is organic N. As total soil nitrogen increases, the quantity of N mineralized from soil organic N increases. At pH's near neutral (pH 7), the microbial conversion of NH_4^+ to nitrate (nitrification) is rapid, and crops generally take up nitrate. In acidic soils (pH < 6), nitrification is slow, and plants with the ability to take up NH_4^+ may have an advantage. Soil pH is also an important factor in the N nutrition of legumes. The survival and activity of *Rhizobium*, the bacteria responsible for N fixation in association with legumes, declines as soil acidity increases. This is the particular concern when attempting to grow alfalfa on soils with pH below 6 as Follett and Schimel (1989) observed that after 16 years of cultivation, total N in the surface 10 cm of soil had decreased by 27%, 32%, and 50% due to no-till, stubble-mulch, and plough treatments, respectively. A soil based approach that attempted to quantify organic N turnover could improve the precision of fertilizer N recommendations and increase N use efficiency (Mulvaney *et al.*, 2005).

Thus, in current study, in open field higher nitrogen content is useful for more N uptake than cultivated field of this weed *Aristolochia bracteolata* Lam. as higher nitrogen content is observed in leaf tissues.

Table 3- Inorganic elements* in soil associated with *Aristolochia bracteolata* Lam. from open and cultivated fields.

Sr.No.	Parameter	Open Field*	Cultivated Field*
1.	Nitrogen	8.14	5.57
2.	Phosphorus	0.36	0.32
3.	Potassium	8.19	6.40
4.	Calcium	272.25	301.84
5.	Magnesium	72.46	64.64
6.	Sulphur	0.16	0.22
7.	Sodium	28.26	24.79
8.	Iron	0.24	0.21
9.	Mangenese	0.45	0.39
10.	Zinc	0.02	0.01
11.	Copper	0.08	0.07

*mg / gm dry matter

Data represents average of three determinations

2) Phosphorus:-

Phosphorus content of soil in open and cultivated field of *Aristolochia bracteolata* Lam. is recorded in Table 3. It is slightly higher in open field (0.36 mg / gm dry matter) than in cultivated field (0.32 mg /gm dry matter).

Soil P exists in various chemical forms including inorganic P (Pi) and organic P (PO^{-}_{4}). These P forms differ in their behavior and fate in soils (Hansen *et al.*, 2004; Turner *et al*., 2007). One unique characteristic of P is its low availability due to slow diffusion and high fixation in soils. (Shen *et al.*,2011). Phosphorus in soils occurs exclusively in the form of orthophosphate with total P concentration usually in the range of 500-500 mg/ Kg. Dry soil Phosphorus associated with organic matter and in mineral soils the proportion of organic Phosphorus lies between 20-8 % of the total Phosphorus. Root-induced chemical and biological changes in the rhizosphere play a vital role in enhancing the bioavailability of soil P (Hinsinger, 2001). More than 90 % of the total Phosphorus is present as insoluble and fixed forms including primary phosphate, minerals, humus, insoluble phosphate of Ca, Fe, Al, P fixed hydrous oxide and silicate minerals. The form and availability of soil phosphorus (P) is also highly pH dependent. Soil microorganisms act as sink and source of phosphorus (P) and mediate key processes in the soil P cycle, e.g., P mineralization and immobilization (Oberson and Joner 2005).

In neutral and acidic soils, phosphate adsorption is the dominant process affecting phosphate availability in plants.

Adsorption of phosphate to soil particles is frequently not an ideal process but rather a combination of adsorption and precipitation (Larsen, 1967). Phosphatase activity and phosphate turnover are much higher in the rhizosphere relative to the bulk soil (Tarafdar and Jungk, 1987).

In our findings low phosphorus content is found in both open and cultivated soil. The availability of this P to plants is a big question because of the alkaline pH of these soils. So in these areas there is general tendency of P deficiency. This fact may be supplemented with low microbial life in these soils.

3) **Potassium:-**

Table 3 depicts potassium content of soil in open and cultivated field associated with *Aristolochia bracteolata* Lam. It was found that K content is higher in open field (8.19 mg / gm dry matter) compared to cultivated field (6.40 mg / gm dry matter).

The average K^+ content of the earth crust is in the order of about 23 g/ Kg. Greatest part of K is bound in primary minerals and in the secondary clay minerals which largely make up of clay fraction of the soil of particle size less than 2 µm. Soils commonly contain over 20,000 ppm of total potassium (McLean and Watson, 1985). Nearly all soil K is in a structural form, and thus not available for plant growth. Plants can use only the exchangeable potassium on the surface of soil particles and potassium dissolved in the soil water (these often amount to less than 100 ppm) (Rehm and Schmitt, 2002).

The release of K from clay minerals is influenced by particle size and chemical composition (Huang, 2005). Soil potassium is classified in three forms according to availability: unavailable, slowly available and readily available or exchangeable (Bhonsle *et al*.,1992). Relationships between clay mineralogy and potassium forms can be used in evaluating potential soil K fertility, prediction of K cycling and plant uptake (Sharply, 1989). The major sources of non-exchangeable K in soils are K-rich 2:1 clay minerals such as micas and vermiculite (Moritsuka *et al.*, 2004).

According to Sparks (1987), slowly available potassium, which is fixed and non-exchangeable, is the form trapped between the layers or sheets of certain kinds of clay minerals; plants can use only very fraction of it during a single growing season.

Minerals' K release to soluble and exchangeable forms and its adsorption by exchange sites depends on the equilibrium between different phases of soil K, which may be affected by factors such as root uptake, fertilizer K applied, soil moisture, soil pH and soil temperature. The greater proportion of high K Clay minerals provides the greater potential K availability. (McLean and Watson, 1985)

According to results obtained higher content in open field sheds light on availability of high K from clay minerals than cultivated soil. However, the overall values of K content fall within the range of adequate availability of soil K to provide normal optimal growth of plant.

4) **Calcium:**

Table 3 gives the idea about calcium content in soils of open and cultivated field associated with *Aristolochia bracteolata* Lam. It was found Ca content is lower in open field (272.25 mg / gm dry matter) and higher in cultivated field (301.84 mg /gm dry matter).These findings are in accordance with the record of $CaCO_3$ in these soils.

The mean Ca concentration of the earth's crust amounts to about 36.4 g/Kg. It is higher than that of most of other nutrients. Ca in soil occurs in various primary minerals. These include the Ca bearing silicates such as feldspar, Ca phosphates and Ca carbonates. They are important in calcareous soils and are usually present as Calcite or dolomite.

In more arid conditions, high Ca content in the upper soil layer may occur in the form of Gypsum ($CaSO_4.2H_2O$).The shallow soils contain appropriate amounts of $CaCO_3$ and for this reason these soils are alkaline in pH.

Ca^+ plays an important role in frequently bridging a carboxylic group of the organic matter with –ve charged a clay mineral surface (Leinwaber, 1995). Soils differ vary widely in their pH and Ca content, particularly in uncultivated soils.

In acidic, humid region soils $Ca^{2+}$and Al^{+3} are dominant because of the CEC (Cation exchange Capacity) while in the neutral and calcareous soils Ca^{2+} occupies the majority of the exchange sites.

Calcareous soils in semi arid and arid region contain 1 to 30 % Ca, predominantly $CaCO_3$, $CaMg(CO_3)_2$ also may be present. Since calcium is a positively charged ion, it is adsorbed in the soil to the negatively charged surface of clay and organic particles. Positively charged ions adsorbed to soil particles are termed "exchangeable ions" because they can be exchanged by other ions present in the soil solution. Soil analysis determines the level of exchangeable calcium ions, and not the total calcium in soil, because the exchangeable calcium is the form which is available to the plants. Calcium that is adsorbed to sodium might cause the soil to crack when dry and swell up when wet.

In present investigation highest amount of Ca is found than other minerals in two soils studied and this may be due to alkaline nature of soils. It is adsorbed to soil particles and helps in stabilizing the soil structure by replacing the adsorbed sodium and prevents damages to soil structure in arid region during water scarcity.

5) **Magnesium:**

Magnesium content of soil associated with open and cultivated field of *Aristolochia bracteolata* Lam. is recorded in Table 3. It is slightly higher in open field (72.46 mg / gm dry matter) than cultivated field (64.64 mg / gm dry matter).

Mg constitutes 1.93 % of the earth's crust, however, total soil Mg content ranges from 0.1 % in coarse humid soils to 4 % in fine textured, arid or semiarid soils formed from high Mg minerals. Mg is present in soil as relatively easily weatherable ferromagnesium minerals, such as biotite, serpentine, hornblende and olivine with average Mg concentration of 130, 250,150, and 240 g / Kg respectively (Dejou,1992).

In arid and semi-arid region, soil may contain larger amounts of Mg and $MgSO_4$. Mg fraction in soils considered as non-exchangable, exchangeable and water soluble form and in equilibrium. Some Mg occurs in association with organic matter in the soil, but this fraction is usually small and less than 1 % of the total soil. Mg among the various divalent cation species Mg^{2+} has the lowest affinity to organic soil complexes (Schnitzer and Skinner,1967). Reducing Mg uptake in many strongly acid soils is caused by high levels of exchangeable Al^{3+} creating Mg deficiency. Mg deficiency can also occur in soil with high Ca : Mg ratio of 10:1 to 15:1.

Cation competition is a consequence of nutrient imbalances in soils. It is commonly known that the uptake of Mg is strongly influenced by the availability of other cations like NH_4, Ca^{2+} and K^+ (Romheld and Kirkby, 2007). Due to high variation in Mg content of the source material the total content of Mg in soils varies considerably between 0.05 and 0.5 % (Maguire and Cowan, 2002). Differences in silicate contents of soils also explain higher Mg contents typically found in clay and silty soils compared to sandy soils. In extreme cases, in soils high in Mg-depleted silicates, mainly chlorites, Mg fixation can occur (Scheffer and Schachtschabel 2002).

Here, in our findings, magnesium content is at second most level in soils associated with *Aristolochia bracteolata* Lam. and justifies alkaline nature of soils. Also, values of Mg are within the adequate range of soil Mg to perform normal plant growth.

6) **Sulphur:**

Table 3 gives idea about sulphur content in soils of open and cultivated field associated with *Aristolochia bracteolata* Lam. It was found that in cultivated field

Sulpher content is (0.16 mg / gm dry matter) and higher in open field (0.22 mg /gm dry matter).

Sulphur occurs in the soil both in inorganic and organic form. Most of the sulphur in soils is found soil in organic matter. However, it is not available to plants in this form. In order to become available to plants, the sulphur must be first released from the organic matter and go through mineralization process which is a result of microbial activity, where sulphur is converted to the sulfate form (SO_4^{-2}), which is readily available to plants. Immobilization of sulphur is the opposite process in which available sulphate is converted back into the organic form.

Soils of dry areas that may have an accumulation of SO^{-4} salts. Most soils contain less than 10 % of the total Sulphur as SO^{-4}. The amount and type of clay, pH, soil organic matter, presence of other anions exert greatest influence on SO_4^{-2} adsorption. Sulphur occurs as coprecipitate $CaCO_3$ –$CaSO_4$ impurity in calcareous soil. The inorganic form of sulphur in soil consists of mainly sulphate. In arid regions soils may accumulate high amount of salts as $CaSO_4$, $MgSO_4$, Na_2SO_4.

The sulphur bacteria (*Beggiatoa, Thiothrix*) oxidize elemental Sulphur to H_2SO_4 in presence of oxygen. Also chemotrophic bacteria *Thiobacillus* oxidizes elemental 'S'. A consequent increase in soil acidity thus occur (Ahmod and Wilson,1992).

Here in soils studied, sulphur content is relatively less as compared to calcium and magnesium as it may be in bound form with Ca and Mg salts thereby increasing soil sodicity.

7) **Sodium:**

Sodium content of soil associated with open and cultivated field of *Aristolochia bracteolata* Lam. is recorded in Table 3. Sodium content is higher in open field (28.26 mg / gm dry matter) than cultivated field (24.79 mg / gm dry matter).

Na content in earth crust is about 2.8% while soils containing 0.1 to 1% Na indicate weathering of Na form Na containing minerals. Very little exchangeable and mineral Na occurs in humid region soils. Na is common in most arid and semi - arid region soils, where it exerts as NaCl, Na_2SO_4, Na_2SO_3 and Na salts that accumulate in specially drained soils contributing to soil salinity and sodicity.

Sodium levels in soil are often reported as the sodium adsorption ratio (SAR). This is a ratio of the amount of cationic (positive) charge contributed to a soil by Sodium, to that contributed by Calcium (Ca) and Magnesium (Mg). The SAR is determined from a water extract of a saturated soil paste. If the SAR is above 13, the soil is classified as sodic.

Soil solution contain between 0.5 and 5 ppm Na^+ in temperate regions. Solution and exchangeable Na^+ varies greatly among soils in arid region soils and if soils irrigated with sodic water, exchangeable Na^+ is greater than K^+ (Havlin *et al.,* 2009).

Na is particularly detrimental because of both its toxic effect on plants and effect on soil structure. When high % of the Cation Exchange Capacity is occupied by Na^+, soil aggregates disperse reducing natural aggregation and soil structure. Water intake is usually poor with sodic soils, especially those high in silt and clay. Poor plant growth and germination are also common in these soils.

In our findings, soil is with sodium adsorption ratio 2.154, pH below 8, and also electrical conductivity less than four reflects that physical soil condition is poor. Even though *Aristolochia bracteolata* Lam. grows well in these soils. Also, in both the fields sodium is greater three times than that of potassium content confirms statements of Havlin *et al*., (2009) that in these soil exchangeable Na is greater than K creating sodic saline condition leading to potassium depreciation.

8) **Iron:**

Table 3 depicts iron content of soil in open and cultivated field associated with *Aristolochia bracteolata* Lam. It was found that it's content in open field is (0.24 mg / gm dry matter) and in cultivated field (0.21 mg /gm dry matter).

Iron is fourth most abundant element, comprising about 5% of the earth's crust. The typical iron concentration in soils ranges from 20,000 to 550,000 mg/kg and occurs predominantly as Fe^{+3} oxides in soils. Ferrous iron is more soluble and bioavailable to plants than ferric iron (Bodek *et al*., 1988). Goethite is the most widespread Fe mineral in soils. Together with other Fe oxides, it greatly influences soil colour which is between yellowish -brown to brown (Allen and Hajek, 1989).

At higher pH levels, Fe^{3+} activity in solution decreases 1000 folds for each pH unit rise. In the high pH range (7 to 9), Fe $(OH_2)^+$, $Fe(OH_3)$, Fe $(OH_4)^-$ are formed. The solubility level reaches a minimum in the pH range between 7.4 to 8.5 (Lindasay and Schwab, 1982). Acidic soils are thus relatively higher in soluble inorganic Fe than calcareous soils where Fe levels can be extremely low.

An important feature of Fe both in soil and plants is the way it readily forms organic complexes or chelates which are called siderophores and are commonly synthesized by bacteria, fungi and plants. They are of crucial importance for the Fe transport in soils and the Fe supply of plants (Masalah *et al.,* 2000).

According to Hoffman and Fletcher (1978) changes in soil properties such as pH or redox status can bring more insoluble iron into these fractions, making it less available to plants. In organic soils, 50 % of the iron was found in the organic plus sulfide fraction.

Thus, from our findings it is clear that in soils studied iron content and its availability is low and this may be due to high pH levels which decrease Fe^{3+} activity.

9) **Manganese:**

Manganese content of soil associated with open and cultivated field of *Aristolochia bracteolata* Lam. is recorded in Table 3. The values of this micronutrient are higher in open field (0.45 mg / gm dry matter) than cultivated field (0.39 mg / gm dry matter).

Manganese occurs in various primary rocks and particularly in ferromagnesian materials. The Mn releases from these rocks by weathering, forms a number of secondary minerals like pyrolusite and mangonite. Total Mn level may differ considerably between soils and are in range from 20 to 3000 µ Mn / g (Gambrell, 1996).

Most available Manganese occurs as exchangeable Manganese. Manganese gets associated with organic matter as various Mn oxides. Soil, high in organic Matter (more than 6%) and near neutral pH (above pH 6.5), may be deficient in Mn. The most important Mn factor in soil is Mn oxides present in amorphous and crystalline form. Mn may be present in di, tri and tetravalent forms, and even in ferromagnesian phyllosilicates may be reduced or oxidized without changing the position in structure (Allen and Hajek, 1989).

Raising soil pH generally decreases the organically bound Mn and increases amorphous and Crystalline Mn form (Zhang *et al.*,1997). It is for this reason that Mn deficiency in plants mainly occurs in soil at high pH.

According to Lindasy (1974) affinity of Mn^{2+} for synthetic chelates is comparatively low and complexed Mn can be easily replaced by Mn^{2+} and present as exchangeable Mn^{2+}. The levels of Mn in soil solution are considerably higher than those of Cu and Zn.

Results obtained in present work clearly indicate that as pH is higher in studied soils, it may lead to deficiency in manganese content. Thus, available Manganese for *Aristolochia bracteolata* Lam. from soil will be also below than average values. However, the plant is not showing any specific Mn deficiency stmptoms.

10) **Zinc:**

Table 3 represents zinc content of soil in open and cultivated field associated with *Aristolochia bracteolata* Lam. It was found that Zn content in open field was (0.02 mg / gm dry matter) and in cultivated field (0.01 mg /gm dry matter).

Zinc content in soil ranges from 17 to 160 µg Zn / g soil (Reed and Martens, 1996). Most of it is present in the lattice structures of primary and secondary minerals (Huang *et al.*, 1989). The radius of Zn^{2+} is very similar to that of Fe^{2+} and Mg^{2+}. Zn solubility is highly dependent on soil pH. It is low when $CaCO_3$ is present for specific adsorption of Zn^{2+} (Donner and Lynn,1989).

Zn interacts with soil organic matter and both soluble and insoluble Zn organic complex are formed. According to Hodgson *et al.*,(1966) an average 60% of the soluble Zn in soil occurs in soluble Zn organic complexes. These are associated with amino acids and fulvic acids as derived from humic acid. Soils originating from basic igneous rocks are high in Zn. Very high levels of Zn may occur in soils which have been affected by wastes and sludge (Juste and Mench, 1992).

Zinc deficiency is most common in soils of the arid regions especially those characterized with high calcium carbonate and low Zn contents (Elsokkary and Log, 1980).

Obtained results in present work are in accordance with work of Elsokkary and Log (1980) as in open and cultivated soil samples also high amount of $CaCO_3$ has been recorded and it indicates low Zn in studied soils.

11) **Copper:**

Copper content of soil associated with open and cultivated field of *Aristolochia bracteolata* Lam. are recorded in Table 3. Cu is in open field is (0.08 mg / gm dry matter) and in cultivated field (0.07 mg / gm dry matter).

Copper occurs in the soil almost in divalent form. Fractions of Cu present in crystal lattice of primary and secondary minerals and high proportion of Cu is bound by soil organic matter. Cu concentrations in soils range from 1 to 40 ppm and average about 9 ppm. Total soil Copper may be 1-2 ppm in deficit soils. Malachite and cupric ferrite are important Cu containing primary minerals and secondary Cu minerals include oxides, carbonates, silicates, sulphates and chlorides. The Cu^{2+} concentration in soil solution governed by Cu adsorption to organic and inorganic soil particles. Thus Cu is very immobile in soils and particularly not leached into deeper soil layers (Mengel and Kirkby, 2006).

Soils with high organic matter (OM) or clay content often have low Cu bioavailability (Gerzabek *et al*., 2006; Lair *et al*., 2007). Cu is the metal forming most strong complexes with organic matter. Kornegay *et al.*, (1976) found Cu to be immobile in soil with high organic matter due to the complexation of Cu by organic matter. He *et al.*, (2011) noted that Cu in organic soils is held tenaciously by the organic matter. Soil parameters, such as pH, organic carbon content, iron and manganese oxide content and total metal content affect the distribution of copper and cadmium among different soil fractions (Fic and Schroter, 1989).

Soil Copper represents Cu^{2+} in the soil solution, decreases sharply on increasing pH whereas the concentration of organic Cu complex in the soil solution is less dependent on soil pH. Higher pH even may promote the dissolution of organic Cu complexes (Mc Bride, 1989).

It is clear from present a study that Copper content is not much affected by soil pH in open and cultivated soils associated with *Aristolochia bracteolata* Lam. Values of

copper content in both soils are within range of optimum levels recorded for normal soils and so this element is not a limiting factor normal plant growth of this weed.

c) Biological Properties:-

In the present work biological properties were studied for the investigation of fungal flora associated with rhozosphere soil of *Aristolochia bracteolata* Lam. from open and cultivated fields. The rhizosphere soils of both the fields were inoculated on PDA medium and after 4-5 days of incubation the fungi were indentified and results are depicted in Table No. 5.

Total 27 colonies alongwith 10 fungal species, 10 genera with 16 x 10^4 colony forming unit / g soil from rhizosphere soil of open field and 24 colonies alongwith 7 fungal genera, 7 species with 9 x 10^4 colony forming unit / g soil form rhizosphere soil of cultivated fields of *Aristolochia bracteolata* Lam. were recorded in Table 4, plate 2, 3 and 4. Fungi *viz. Trichoderma viridae, Penicillium notatum, Aspergillus fumigatus, Mucor indicus, Rhizopus stolonifer, Humicola fuscorata, Fusarium* sp., *Stachybotrys* sp., *Cladosporium* sp., and *Acrimonium* Sp. were recorded from rhizosphere soil of open field. While in cultivated fields *Penicillium notatum, Aspergillus fumigatus* and *Alternaria alternata* along with *Cladosporium sphaerospermum, Humicola fuscorata, Acrimonium* sp. *Gleomastix* sp. were recorded. In both the fields *Penicillium notatum, Aspergillus fumigatus, Cladosporium sphaerospermum, Humicola fuscorata* were most commonly recorded.

Most of the work has been carried out on fungal flora associated with rhizosphere soil of various plant species. In arid land ecosystems biological activity is constrained by episodic water availability, high soil pH, extreme soil surface temperature and intense solar radiation. These abiotic characteristics create patchy distributions of grass tussocks and biological soil crust (BSC) and develop selected microbial communities that are compositionally and functionally distinct from those of mesic ecosystems (Collins *et al.,* 2008). Root exudates are known to attract and maintain symbiotic fungal communities that form mutualistic associations with plant roots and colonize surrounding soil (Morgan *et al*., 2005).

Descriptions of fungal components of arid ecosystems based on culture-based and molecular techniques showed that dark-septate fungi (DSF) are most dominate (Bates

Table 4: Biological properties of rhizosphere soils from open and cultivated field associated with *Aristolochia bracteolata* Lam.

	Open Field	Cultivated Field
Total no. of colonies	27	24
Colony forming units (CFU) / g soil	16×10^4	9×10^4

Data represents average of three determinations

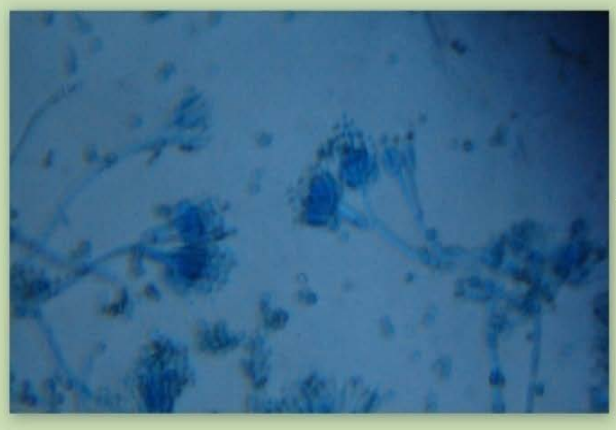
Plate 2 - Colonies of soil fungi isolated from Open fields of rhizosphere soil of *Aristolochia bracteolata* Lam.

Plate 3 - Colonies of soil fungi isolated from cultivated fields of rhizosphere soil of *Aristolochia bracteolata* Lam.

Table 5: Soil fungi isolated from rhizosphere soils from open and cultivated field associated with *Aristolochia bracteolata* Lam.

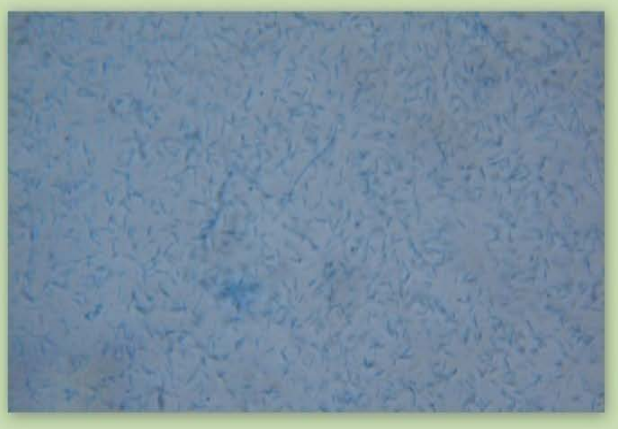
	Open Field	Cultivated Field
Name of fungi	*Trichoderma viridae*	*Penicillium notatum*
	Penicillium notatum	*Aspergillus fumigatus*
	Aspergillus fumigatus	*Alternaria alternate*
	Mucor indicus,	*Cladosporium sphaerospermum*
	Rhizopus stolonifer	*Humicola* sp.
	Humicola fuscorata	*Acrimonium* sp.
	Fusarium sp.	*Gleomastix* sp.
	Stachybotrys sp.	
	Cladosporium sp.	
	Acrimonium Sp	

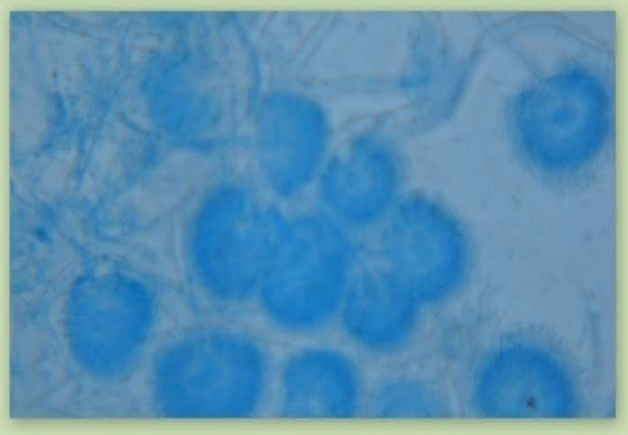
Plate 4 - Soil fungi isolated from rhizosphere soil of *Aristolochia bracteolata* Lam.



Penicillum sp. isolated from rhizosphere soil of *Aristolochia bracteolata* Lam.

Alternaria sp.isolated from rhizosphere soil of *Aristolochia bracteolata* Lam.





Mucor indicus isolated from rhizosphere soil of *Aristolochia bracteolata* Lam.

and Garcia-Pichel, 2008). These phylogenetically diverse fungi have pigmented hyphae due to the presence of fungal melanin that provides resistance to UV radiation, drought and high temperatures (Zak, 2005).

Unlike boreal and temperate forests, which are dominated by Basidiomycota the soils and plant roots of semi arid grasslands are colonized predominantly by Ascomycota, including a large number of DSF i.e. Dark Septate Fungi (Allison *et al.*,2007). Fungi *viz*. *Alternaria, Phoma, Bilpolaris, Aureobasium* are most common genera from arid ecosystem was reported by many workers from different countries like States and Christensen (2001) reported from Utah and Wyoming, USA, Durell and Shields (1960) from Nevada and Death valley, USA, Grishkan *et al.*,(2006) from Negev Desert, Israel. The great abundance of Pleosporales and DSF in arid grasslands may be explained by the presence of melanin pigments in their hyphae. Fungal melanins have been associated with mitigating environmental stresses including high temperatures, high UV radiation and extended drought (Bell and Wheeler, 1986). DSF may be the key component of arid environments specifically because they are adapted to high temperatures and high salt concentrations (States *et al.,* 2001). Andrea *et al.,* (2011) studied diversity and distribution of soil fungal communities in a semiarid grassland and observed the abundance of *Alternaria, Fusarium, Cochliobolus, Bipolaris, Phaeosphaeria, Leptosphaeria, Preussia* fungi.

According to Zak *et al.,* (2003) interaction between plants and Arbuscular Mycorrhizal fungi (AMF) and Dark Septate Endophytes (DSEs) provide more efficient use of the available resources to plants. Distribution of Arbuscular Mycorrhizal (AM) fungi association with ten important medicinal plants of Tamil Nadu including *Aristolochia bracteolata* Lam. were studied by Mohan *et al.,* (2005). They noticed maximum percent root colonization and soil spore population of AM fungi in *Ocimum sanctum* and *Centella asiatica*. While low percent colonization and soil spore population recorded in *Cichorium intybus*. Most dominant fungus was *Glomus* which had infected ten different species of plants.

Sundar *et al.,* (2011) studied association of arbuscular mycorrhizal fungi from three medicinally important plants *viz. Eclipta prostrata, Indigofera aspalathoides* and *Indigofera tinctoria* from different localities of Kanyakumari. They reported total 21 AM

fungal species with high fungal species richness, colorization percentage and Shannon index in two *Indigofera* sp. and low in *E. prostrata.*

Santos *et al.,* (2013) evaluated 36 weed species for symbiotic fungal association. They observed morphological types of arbuscular and coiled hyphae. In most of the plants association of Dark Septate Endophytic fungi were observed. Similarly Kokare and Shinde (2012) studied arbuscular mycorrhizal fungi associated with five xerophytes *viz. Argemone mexicana* Linn., *Aristolochia bracteolata* Lam., *Chlorophytum tuberosum* Baker., *Scilla indica* Baker., *Tribulus terestris* Linn. from Ghodnadi river of Pune district. In their study dominant fungi was *Glomus* with 13 species followed by *Acaulospora* with four species, two species of *Scutellospora* and one species of *Gigaspora* and *Sclerocystis.* Arbuscular mycorrhiza (AM) and dark septate endophytes association in twenty medicinal and aromatic plants of Guwahati were studied by Sharma and Zha (2012), they observed that all plant species having AM fungal association but only 12 species among them have DSE colonization. Recently Muthuraj *et al*., (2014) studied mycorrhizal diversity of 46 medicinal plants of Madayipara hills, Kannur, Kerala. They noticed 35 AM fungal species belong to four genera *viz. Aculospora, Gigaspora, Glomus and Scutelospora.*

From the present study it may be concluded that, the arid land of Baramati region is rich with fungal flora belonging to order Pleosporales and DSF (Dark Septate Fungi) like *Alternaria* and *Fusarium* spp. which are adapted to high temperature and high salt conditions.

B) Studies of *Aristolochia bracteolata* Lam.

a) Growth analysis

Growth of plant can be analyzed by measuring a variety of growth parameters. Growth and development of a plant can be studied by making periodic observations of plant root length, shoot length number of leaves per plant, fresh weight, dry weight, Average leaf area of plant.

Growth analysis is an important tool for studying very complex interaction between plant and the environment. This interaction helps understanding the critical phase in the life cycle of the plant.

Table 6- Some growth parameters in mature *Aristolochia bracteolata* Lam.plant

Sr.No.	Parameter	Findings
1	Shoot length	11.12 ± 1.61cms
2	Root length	11.74 ± 0.23cms
3	No. of leaves / Plant	19.7 ± 1.85
4	Average leaf area	4.47 ± 1.33cm^2

Data represents average of ten determinations

± indicates Standard deviation

Table 7- Yield data of *Aristolochia bracteolata* Lam. plant

Sr.No.	Parameter	Findings
1	Total Pods / Plant	20 ± 0.37
2	Total Seeds / pod	89 ± 0.6
3	Total seeds / plant	1100 ± 2.85
4	100 seed weight	0.535 ± 0.01gm

Data represents average of ten determinations

± indicates Standard deviation

Some growth parameters of *A.bracteolata* Lam. are represented in Table 6. It is clear from Table 6 that this plant at maturity has an average 11.12 cm shoot length and 11.74 cm root length. The plant branches profusely from the base. The underground erect stem is fleshy with several perennial buds about 20 leaves are present totally with 4.47 cm^2 average leaf area.

The reproductive potentiality of the weed is depicted in Table 7. The plant has an average 20 pods on the plant even though large no. of seeds is present per pod. Average seed output per plant is quite high 1100 seeds/plant. Weight of 100 seeds is 0.535 mg indicates light weight nature of seed.

Earlier in ecophysiological studies in some weed species around Baramati region Murumkar (1993) observed that in *A. bracteolata* Lam. shoot length was 18.6 cms and 23.2 cm root length with 28 leaves per plant and 14.85 cm^2 average leaf area alongwith average seed output was less and 100 seed weight was 0.068 mg.

Continuity and spread of the species is directly related with the existing quota of seed bank (Hume and Archibold, 1986) and its seasonal emergence (Hakansson, 1983).

b) Leaf Water Relations-

Water relations in leaves of *A.bracteolata* Lam. are presented in Table 8, Figure 3 and 4. It can be observed from the Table 8 that moisture content is highest in leaf (73.81 %) followed by stem (68.93 %) whereas lowest in root (44.04 %). Same Table gives idea about succulence index. It is again high in stem (3.92) followed by leaf (3.27) and low content in roots (1.82). High moisture content is because of high mucilage in leaf mesophyll tissue and values falls within the range of CAM plants. More Succulence in this plant is because of fleshy shoot part and stores high amount of water (Murumkar, 1993).

Water retention capacity is of much significance. This can be attributed to the bound form of water in the vacuoles. Walker and Miller (1986) have used the criterion of higher leaf water content to determine the resistance towards drought. According to them higher values of leaf water content represent drought resistance while lower values towards susceptibility. It is postulated that plant development is controlled primarily by combined and interacting effects of growth, osmotic gradient and transpiration on uptake

Table 8- Moisture content (%) and Succulence Index (SI) in different plant parts of *Aristolochia bracteolata* Lam.

Plant Part	Moisture content (%)	Succulence Index (SI)
leaf	73.81 ± 0.62	3.92 ± 0.07
Stem	68.93± 1.26	3.27 ± 0.45
Root	44.04 ± 4.87	1.82 ± 0.27

Data represents average of ten replications.

± indicates Standard deviation

Fig. 3- Moisture content (%) in different plant parts of *Aristolochia bracteolata* Lam.

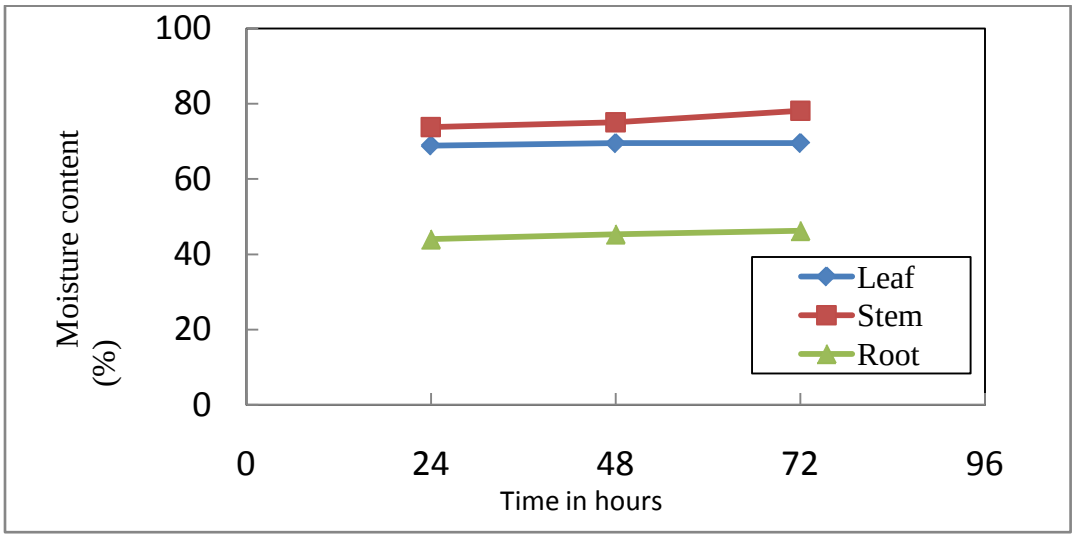
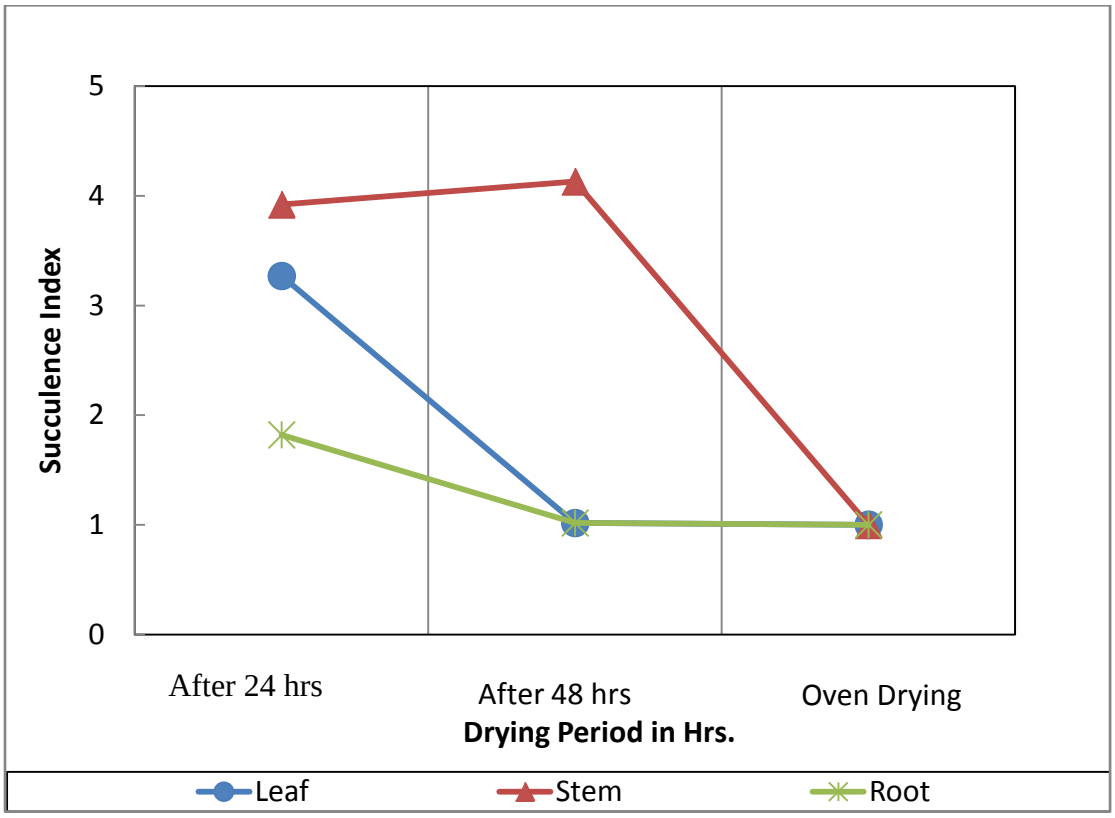


Fig. 4- Succulence Index in different plant parts of *Aristolochia bracteolata* Lam.



distribution and loss of water (Mc.Inthyre,1985). Water is the medium by which mineral nutrients enter and are translocated through plant. It is also necessary to maintain the leaf turgidity without which photosynthesis and many other metabolic processes cannot operate properly. The amount of desiccation that can be tolerated and ability to survive and to recover from an unfavourable water balance varies from species to species (Birot, 1965).

A.bracteolata Lam. adapts to arid conditions where water is limited. The weed maintains water content of its tissue at a constant and relatively high level by means of one or more physiological and anatomical features. These adaptations reduce excessive water loss, facilitate absorption by developing root system or permit water conservation in plant tissues.

c. Seasonal variations in leaf water Status in *A. bracteolata* Lam.-

Table 9 represents findings of seasonal variations in parameters of leaf water status in leaves of *Aristolochia bracteolata* Lam. Perusal of the data revealed that leaf showed highest moisture content in summer season (87.43 %) followed by monsoon and winter season. Also mesophyll succulence index is high in summer season than monsoon and winter season but exhibit remarkable high values which fall within the range of 1.5 to 13.0 reported of CAM plants (Kluge and Ting, 1978). Same Table (Table 9) depicts surface expansion values for this weed it is again high in summer season than monsoon and winter season. These values are also in accordance with those reported for CAM plants (Walter,1926). Osmotic potential is also in accordance with above parameters.

High moisture content is because of high mucilage in leaf mesophyll tissue and moisture content is maintained by development of leaf succulence index in *Aristolochia*. All these above mentioned features point clearly towards CAM nature of this plant (Murumkar, 1993). CAM plants are more efficient in maintaining the leaf turgor through water balance as well through adapted mode of photosynthesis (Osmond, 1978). From Table 9 it is evident that *Aristolochia* has successfully adapted the strategy of maintaining water balance under unfavourable condition like summer season of this area. This justifies the survival and dominance of this weed species in drought prone area in summer season.

Table 9 - Seasonal variations in various Parameters of Leaf Water Status in leaves of *Aristolochia bracteolata* Lam.

Sr. No	Parameter	Summer	Monsoon	Winter
1	Moisture content (%)	87.43 ± 0.02	69.14 ± 0.01	56.80 ± 0.04
2	Succulence Index $g.g^{-1}$	7.92 ± 0.30	5.14 ± 0.17	3.69 ± 0.21
3	Mesophyll Succulence index(sm)g^{-1}	4.98 * ± 0.43	3.56 * ± 0.27	2.24 * ± 0.45
4	Surface Expansion m^2kg^{-1}	3.15 * ± 0.49	2.78 *± 0.12	1.52 * ± 0.25
5	Osmotic Potential (Atm. pressure into bars)	-1.27 ± 0.03^{ns}	-1.15 ± 0.08^{ns}	-0.91 ± 0.05^{ns}

* : significant at $p < 0.05$

ns : Values are non-significant .

d) Germination Studies

Germination process is physiological event depends on the quality of seeds and germination conditions, as the supply of water and oxygen, temperature, light and adequate substrate. The response of seed to these factors varies with the species (Mayer and Poljakoff-Mayber, 1975).

The germination of seeds of *Aristolochia* is found to be very poor. Also, seeds showed seed dormancy. So when subjected to various treatments to break seed dormany, seeds showed diverse response. Results of different treatments to the *A.bracteolata* Lam seeds and it's effect on germination is presented in Table 10. Mechanical scarification treatment showed 90% germination. Whereas hot water treatment showed 40% germination. While stratification treatment showed germination upto (60%).

According to Adams *et al.*(2005) a long period of cold stratifying treatment was required for growth of underdeveloped embryos and seed germination in *A.californica*, whereas embryo grew and seeds germinated in the *A. macrophylla, A. tomentosa, A. manshuriensis* of warm temperatures either before or after they were cold stratified.

Viability of seeds looses with increase in storage time. The viability of a seed is a lifespan of a particular seed during which it is able to germinate and which varies from species to species.

Aristolochia esperanzae seeds had their germination inhibited in the absence of light which characterised them as positive photoblastic. The presence of light and temperatures 25^0 C and 30^0C favoured seed germination and normal seedling formation (Maekawa, 2010). Scalon and his co-workers (2007) reported that seeds of *A. triangular* did not germinate at 15^0 C, which indicates the sensitivity of seeds of genus *A. triangular* at lower temperature of that 20^0 C.

Medicinal species of cerrado, normally are indifferent to light and germinate both with and without light (Albuqueaque *et al.*, 2003). Recently Alves-Da-Silva (2011) postulated that Aristolochicceae has been described as seeds with underdeveloped embryos and morphological or morphophysiological dormancy and concluded that no particular treatment was required for embryo development, in *Aristolochia gelata* seeds but seeds in the population that continued to germinate after 1 month were probably in

Table 10– Results Showing various treatments of dormancy in *Aristolochia bracteolata* Lam.

Sr. No.	Type of Treatment	% of germination
1	Mechanical scarification	90
2	Hot water treatment	40
3	Stratification	60

Data represents average of fifteen determinations

various states of non-deep, simple morpho-physiological dormancy. Increased germination in nitrate treatments and light requirement for germination could prevent germination under unsuitable environmental conditions and be a strategy to increase seedling establishment in *A. gelata* seeds.

Results obtained and from above discussion it justifies that hard seed coat may cause dormancy due to impermeability of testa to water. Mechanical abrasion to the hard seed coat can remove the so called dormancy in this weed as higher germination is observed in mechanical scarification treatment.

e) Inorganic Elements-

There are now 17 elements which are considered essential for plants to complete their life cycle. These essential plant nutrients are divided into two categories; macronutrients and micronutrients. Macronutrients include carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S). Micronutrients are zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), boron (B), molybdenum (Mo), chlorine (Cl) and nickel (Ni) (Epstein, 1972). Although silicon (Si) is not essential, it is considered as a beneficial plant nutrient. These plant nutrients are not only required for better plant growth and development, but also helpful to alleviate different kinds of abiotic stresses like drought stress. Plants have developed a wide range of adaptive and resistance mechanisms to maintain productivity and ensure survival under a variety of environmental stress conditions. Increasing evidence suggests that mineral-nutrient status of plant plays a critical role in increasing plant resistance to environmental stress factors (Hampe and Marschner, 1982).The essential nutrients required by higher plants are exclusively of inorganic nature. Macronutrients are found and needed in plants in relatively higher amount than micronutrients. But, both are essential for almost all metabolic processes. The mineral nutrition is an important aspect of plant growth that governs the productivity of all plants.

Most micronutrients are predominantly the constituents of enzyme molecules and are essential only in small amounts. In contrast, the macronutrients are either the constituents of organic compounds, such as proteins, polysaccharides, lipids and nucleic acids. These differences in function are reflected in average concentration of mineral nutrients in plant shoots that are sufficient for adequate growth (Marschner, 1986). The

values are considerably varying depending on plant species, plant age and concentration of other mineral elements. The main factor controlling the mineral content in plants is the specific, genetically fixed nutrient uptake potential for the different mineral nutrients (Mengel and Kirkby, 1980).

If soil has low concentration of a particular element, plant shows deficiency symptoms. There are various factors like pH, composition of elements of soil, climate and heavy rain fall responsible for developing deficiency of mineral elements in plants (Gauch, 1957). Mineral deficient plants show different symptoms like chlorosis, malformation, necrosis, dwarfism (reduced growth), dieback and lastly death of plant. Such deficiencies and their symptoms on plant metabolism are well discussed by Epstein (1972). Also the unfavorable environmental conditions affect the uptake, transport and distribution of plant nutrients within the plant body.

The status of mineral elements in different parts like leaf, stem and roots of mature *A. bracteolata* Lam. is presented in Table 11.

1. Nitrogen

The Nitrogen content in leaf, stem, root and seed of *A. bracteolata* Lam. is shown in the Table 11. In *Aristolochia* highest nitrogen content is recorded in leaf (420.50mg / gm) followed by stem (197.04 mg/gm) and lowest value is recorded in roots (137.79 mg /gm).While in seed it is recorded to (261.30 mg/gm).

Nitrogen (N) is the most important mineral nutrient for plants. Since plants require the greatest quantity of N of all the mineral elements, deficiency of N is a limiting factor for plant growth (Krouk *et al.,* 2010). Dry plant material contains about 20-44 mg N/Kg. This appears rather low in comparison with the C concentration which is in order of about 400 mg/Kg dry matter. Nevertheless, N is an indispensible elementary constituent of numerous organic compounds of general importance viz. amino acids, proteins, nucleic acids, and compounds of secondary plant metabolism such as alkaloids (Mengel and Kirby, 2006). N is required for the synthesis of amino acids, proteins, chlorophyll, nucleic acids, lipids, and a variety of other metabolites containing N in their structure (Tabuchi *et al.,* 2007). Tschoep *et al.,* (2009) reported that as nitrogen is the most crucial element for plant growth and metabolism, measuring metabolite levels using metabolomic techniques provides basic information about biological responses to

Table 11 -Inorganic element content in different plant parts of *Aristolochia bracteolata* Lam.

Inorganic Element	Root*	Stem*	Leaf*	Seed*
Nitrogen	137.79	197.04	420.5	261.3
Phosphorus	38.74	41.97	58.12	135.6
Potassium	242.98	62.97	61.39	396.44
Calcium	184.64	56.64	79.1	20.46
Magnesium	12.35	18.52	16.46	19.75
Sulphur	31.19	77.96	11.23	68.61
Sodium	39.15	71.75	45.67	41.08
Iron	0.36	0.93	2.17	0.18
Mangenese	0.84	0.96	0.76	1.53
Zinc	0.41	0.31	0.06	0.17
Copper	0.01	0.03	0.02	0.01

*mg / gm dry matter

Data represents average of three determinations

physiological or environmental changes triggered by nitrogen status. N assimilation by plants requires NO_3^- absorption, its reduction and subsequent conversion to NO_2^-, and NH_4^+, respectively, and the final NH_4^+ incorporation into organic compounds (Stitt, 1999).

Marschner, (1995) states that out of total nutrients observed 80% contribution is of N in plants and suggested that nitrogen application, where light is not limiting, increases antioxidative defence mechanism. It is taken up by plants both in organic (Urea as foliar spray) and inorganic Ammonium (NH_4^+) and Nitrate (NO_3^-) as soil application forms. Lawlor, (2002) reported that metabolic processes based on proteins, leading to increase in vegetative and reproductive growth and yield, are totally dependent upon adequate supply of Nitrogen.

Our results indicate that *Aristolochia bracteolata* Lam. is efficient in absorption of N and its translocation to shoot as more N content is recorded in leaf followed by stem. The accumulation of novel alkaloid Aristolochic acid in leaves and stem also justifies N accumulation in shoot (Table 11). Obviously, N efficiency of this weed may be one of the adaptive features towards survival in unfavorable drought conditions.

2. Phosphorus-

The phosphorus content in leaf, stem, root and seed of *A. bracteolata* Lam. is presented in the Table 11. It is well evident from the Table that phosphorus content is highest in leaf (58.12 mg/gm) followed by Stem (41.97 mg/gm), roots (38.74 mg/gm). Whereas in seeds it is 135.60 mg / gm.

Phosphorus (P) is the second nutritional element after nitrogen that limits plant growth, having a concentration of about 0.2% of the total plant dry weight (Raghothama, 1999). Phosphorus is component in many molecules and the principle element involved in plant energy processes like nucleic acids, phospholipids and ATP that participate in basic plant processes (Schachtman *et al.*, 1998). It is a constituent of nucleic acid, phospholipids, Phosper – proteins, dinucleotide and adenosine triphosphate. Hence, it is required for processes including the storage and transfer of energy, photosynthesis, and regulation of some enzymes and transport of somecarbohydrates (Hu and Schmidhalter, 2001). In its inorganic form (Pi) it is involved in the control of many enzymatic reactions, which regulate different metabolic processes (Theodorou & Plaxton, 1993). The positive

effect of P on plant growth under drought have been attributed to an increase in stomatal conductance (Bruck *et al.*,2000), Photosynthesis (Ackerson,1985). Phosphorus also maintains the cell turgidity by maintaining the high leaf water potential which in turn increases the stomatal conductance.While P deficiency includes the reduction in leaf number (Lynch *et al*., 1991) and loss of photosynthetic efficiency (Lauer *et al.*, 1989).

As per expectation, highest P content is recorded in leaf followed by stem i.e. in shoot part in *Aristolochia*. This gives clear idea that this weed is receiving adequate quantity of P from soil and thereby maintaining its normal metabolic activity.

3. Potassium:

The Potassium content in leaf, stem, root and seed of *A. bracteolata* Lam. is presented in the Table 11. It is well evident from the Table that maximum potassium content is found in stem (62.97 mg / gm dry matter) followed by leaf (61.39 mg / gm dry matter), while the lowest value is recorded in root (242.98 mg / gm) amongst vegetative parts. In seed, potassium content is 396.44 mg / gm.

Potassium plays a significant role in plant growth and developmental processes such as photosynthesis (Peoples and Koch, 1979), translocation of proteins and carbohydrates (Marschner, 1997), activation of enzymes (Suelter, 1970), stomatal movement (Humble and Raschke, 1971), nyctinastic and seismonastic movements, cell extension, etc. Potassium is indispensable for plant growth. Plants require 1% potassium for their optimal growth (Epstein, 1972). It plays a key role in the process of stomatal behaviour, assimilate translocation and osmoregulation. It works as a cofactor of enzymes involved in translocation and stomatal opening. It is involved in a wide range of metabolic activities such as carbohydrate metabolism, glycolysis, phosphorylation and adenine biosynthesis in plants.

Its optimum level in plants is 0.3-6% of dry matter. It is required as cofactor for more than 400 enzymes, maintains electonutrality of cell and has a major role in stomatal movement. It also plays important role in several physiological processes such as photosynthesis, translocation of photosynthetes into sink organs, maintenance of enzymes activation and reducing excess uptake of ions such as Na and Fe in saline and flooded soil (Mengel and Kirby, 2006).

The protective role of K in plants suffering from drought stress by maintenance of high pH in a stroma and against the photooxidative damage to chloroplast was reported by (Cakmak, 2005). Possible mechanism helpful in minimizing detrimental effects of drought by improving water use efficiency in crop plants with K nutrition has been described by Waraich *et al.* (2011). Potassium maintains the osmotic potential and turgor of the cells (Lindhauer, 1989) and regulates the stomatal functioning under water stress conditions (Kant and Kafkafi, 2002).

According to Flower *et al.*, (1986), potassium is unevenly distributed between the vacuole and cytoplasm in plant cells and especially, in halophytes it is mainly accumulated in the cytoplasm and is compartmentalized in the vacuoles. The uptake of potassium is highly selective and closely coupled to metabolic activity.

As potassium is highly mobile element (Mengel and Krikby, 1980), in plants its deficiency is usually observed in older leaves, which become chlorotic and develop necrotic patches. It's deficiency causes reduction in nitrate reductase activity, disturbance of protein metabolism, accumulation of amino acids and soluble organic nitrogenous compounds.

From the present results it is revealed that potassium accumulated more in stem tissue of *A. bracteolata* Lam. thereby helping in transport of solutes, maintains osmotic potential and helps leaves for osmotic adjustment under environmental limiting factors.

4. Calcium

The calcium content in leaf, stem, root and seed of *A. bracteolata* Lam. is presented in the Table 11. It is well evident from the Table that level of Calcium is higher in leaves (79.10 mg/gm) and lowest in roots (184.64 mg / gm).While in seeds it is 20.46 mg / gm).

Calcium was not only considered important for cell wall structure, but since the recent discovery of calmodulin, Ca is not just a macronutrient but also a major controller of plant metabolism and developement (Poovaiah and Reddy, 2000). Calcium is considered to play an important role in mediating stress response during injury recovery from osmoregulation and acclamatization to stress. It has been suggested that Ca is necessary for recovery from drought by activating the plasma membrane enzyme ATPase which is required to pump back the nutrients that were lost in cell damage (Palta, 2000).

Since dehydration is the common denominator, Ca has also a role to play in freeze injury tolerance. Possible mechanism to minimize detrimental effects of drought in crop plants is by improving Ca nutrition. Calcium has a very important role in the maintenance of cell structure, works as an osmoticum within vacuoles (Dodd *et al.,* 2010).

As most plant Ca is stored in leaves and root to shoot transfer of Ca^{2+} has been elaborated at length (Karley and White, 2009). The leaf vacuole is widely considered to be the major Ca store in plant tissues but, unlike the cytoplasm, (Ca^{2+}) in vacuole varies considerably between cell types (White and Broadley, 2003). Calcium uptake and distribution at the whole-plant level is influenced by water flow to transpiring organs and by the relative rate of Ca^{2+} uptake along the transport pathway (McLaughlin and Wimmer, 1999). It is known that (Ca^{2+}) apo adjacent to the guard cell will regulate stomatal aperture, in response to low temperature stress (Wilkinson *et al.,* 2001) and it has been proposed that this may involve an interaction with AQPs which are responsible for finely regulating water flows across plant membranes (Tyerman *et al.,* 1999) and in the guard cells (Yang *et al.,* 2006).

In present findings also more accumulation of Ca in leaf tissue of *Aristolochia bracteolata* Lam. is observed. It may take part in regulation of water flow across plant membranes thereby helps in stomatal movement to reduce water loss and controlling cell membrane from injury recovery under unfavorable conditions.

5. Magnesium

The magnesium content in leaf, stem, root and seed of *A. bracteolata* Lam. is presented in the Table 11. From the Table it is well evident that maximum magnesium content is found in stem (18.52 mg / gm) followed by leaf (16.46 mg/g), and lowest in roots (12.35 mg/gm). In seeds it is recorded upto (19.75 mg / gm).

Magnesium, eventhough taken up by the plants in lower quantities, is very essential for several physiological processes. Magnesium is upto 0.05-0.7% of dry matter of plants. Epstein (1972) has given 0.2 % dry matter as a critical concentration for this element. This divalent cation forms central core of nucleus of i.e. tetrapyrole ring of chlorophyll molecule as well as acts as cofactor in almost all enzymes involved in phosphorylation process. It is well established that this ion plays a key role in activation of several enzymes like RUBPcase, ATPase (Kawasaki *et al.,* 1979) and alkaline

inorganic pyrophosphatase (Mukherjee and Pal, 1983). Bernstein (1975) had postulated that the ability to absorb nutritionally adequate levels of Ca^{2+} and Mg^{+} from the soil determines tolerance of species for a particular salt.

In all ribozymes, which are metalloenzymes that require divalent cations, require high Mg^{2+} concentration for binding the tRNA strand to the ribosomes (Ahsen and Nollerr,1995). From this, it is also obvious that an inadequate supply with Mg^{2+} has a detrimental impact on polypeptide synthesis and thus on protein formation. This conclusion is in agreement with earlier findings of Haedar and Mengal (1969) who reported that in Mg deficient plants the proportion of protein-N decreased and that of non-protein –N decreased.

The data of Grimme *et al*.(1974) showed that high Mg Concentrations may occur in plants supplied with a low level of K^{+} nutrition. Not only the uptake but also the translocation of Mg^{2+} from roots to upper parts of plants can be restricted by K^{+} and Ca^{2+} (Schimanski,1981).

In *Aristolochia bracteolta* Lam. Magnesium content is more in stem as well as leaves and is minimum in roots. High Mg content in shoot part indicates the normal healthy activity of this weed and involvement in K translocation. Levels of Mg in this weed fall within range of normality and effective metabolic turnover. It helps for synthesis of other nitrogen containing compounds like aristolochic acid which is the chief constituent of secondary metabolite of this plant.

6. Sulphur

The sulphur content in leaf, stem and root of *A. bracteolata* Lam. is presented in the Table 11. From the Table it is well depicted that Sulphur content is highest in leaf (11.23 mg/gm) and while it is lowest in root (31.19 mg /gm). Seeds contain 68.61 mg/ gm sulphur.

Sulphur is an important constituent of amino acids like cysteine, methionine and thus proteins. It is a constituent of lipolic acid enzyme, thyminepyrophosphate, glutathione, biotin, adenosine 5 sulphophosphate and 3- phosphoadenosine. It is utilized in the form of sulphate ions (SO_4^{-}). Sulphur may also be absorbed as sulphur dioxide (SO_2) from the air through leaves in areas where the atmosphere is enriched with sulphur compounds. It is not readily available from Wettable sulphur which is in the form of

elemental sulphur. It is essential for nodule formation on legume roots (Singh and Bajpai, 1982). Sulphur is present in oil and aromatic compounds responsible for the characteristic odour of plants such as garlic and onion.

Sulphur content of common farm crops was considerable, cereal grains containing about half as much sulphur as phosphorus and legume hays sometimes more sulphur than phosphorus, while the Cruciferae, such as cabbage, turnips, etc., may contain two to three times as much sulphur as phosphorus (Hart and Peterson, 1911). It has been urged by Hopkins (1911) that the high sulphur content of plants does not represent their needs, but merely shows the superabundance of sulphates in the soil water, with an extraordinary consumption by the plant. This may apply to the stem and roots of plants, but not to the seed. A cabbage crop that absorbs 100 pounds per acre of sulphur trioxide makes use of this material in a different way from a potato crop which absorbs but 11 pounds of sulphur trioxide (Hart and Tottingham, 1915).

In *Aristolochia* sulphur content is higher in leaves as well as stem, thus in shoot system while it is minimum in roots. The insecticidal property and its ethnobotanical characteristic nature as 'Wormkiller' reflect its chemical constitution rich in sulphur. The bitterness attributed to this weed may be also the effect of high sulphur content. Aristolochic acid which may contains sulphur is accumulated in shoots of this plant and role of sulphur in synthesis of this acid will be worth to trace.

7. Sodium-

The important micronutrient sodium in leaf, stem and root of *A. bracteolata* Lam. is presented in the Table 11. The pattern of accumulation of Sodium in different parts of *A.bracteolata* Lam. is highest in stem (77.96 mg/gm) > leaf (45.67 mg/gm) > root (39.15 mg/gm).The seeds show moderate amount of Na (41.08 mg/gm).

According to Pitman, (1976) sodium is successful competitor for potassium during ion uptake. Role of sodium in maintaining favorable water balance has been suggested by Rains (1972). Kylin (1973) reported stimulation of ATPase in presence of sodium. Even when potassium supply is limited micronutrient sodium stimulates growth through enhanced cell expansion. It is also required in maintaining membrane integrity (Brownell, 1979). Under sodium deficiency plants exhibit chlorosis and necrosis or even

fail to form flower. Many C_3 species also benefit from exposure to low levels of sodium ions.

According to Chirputkar (1969) adequate level of sodium for glycophytes is 0.6 to 1.4% dry wt. The range of sodium in glycophytes as given by Gauch (1972) is 0.1 to 1.4% dry wt. In crop plants like sugarcane, 0.11% sodium content was recorded by Nimbalkar (1973), in finger millet 0.09% (Chavan, 1980), in chick pea 0.009 % (Murumkar,1986).

Sodium is always found in adequate quantities in arid soils. Sodium content in *Aristolochia* is within the range of glycophytes as per present findings. However, its high values in leaf indicate the adaptive mechanism acquired by this weed to survive in arid region by maintaining water integrity through succulence.

8. Iron

The another micronutrient, iron content in leaf, stem and root of *A. bracteolata* Lam. is presented in the Table 11. Iron is mostly accumulated in leaf tissues (2.17 mg /gm) followed by stem and roots tissue (0.36 mg/ gm). Whereas in seed Fe content is very less (0.18 mg/gm).

Iron concentration of green plant tissue is low in comparison with the macronutrient and generally in the order of 50 to 100 µg Fe/g dry matter. Though considered as microelement, Fe is very essential for enzyme systems where it functions as prosthetic group haem or haemin. Iron is involved in photooxidation reduction reactions via ferridoxin as well as in chlorophyll synthesis (Spiller and Terry, 1980). Ferrodoxin contains 10 atoms of iron per molecule of which 8 are probably attached to sulphahydryl groups in ferrous form. There are evidences for involvement of ferrous irons in condensation of succinic acid and glycerine to form aminolaevulinic acid (ALA) which further condenses to form pyrrole group. Ferrodoxin is also involved in the reduction of nitrite to ammonia (Losada *et al.,* 1963) and in nitrogen fixation by bacteria (Emerich *et al*.,1983). An important enzyme system in nitrogen fixation dinitrogenase contains a cofactor composed of molybdenum, iron and Sulphur in the proportion of 1:8:6 (Shah and Brill, 1977). Another component of nitrogenese, dinitrogenase reductase contains four atoms of Fe and four acid labile sulphur atoms per molecule (Emerich and

Evans, 1980). There are reports of both iron deficiency as well as iron toxicity in crop plants raised under different field conditions (Mehrotra *et al.,* 1976)

According to Maas *et al*. (1972) increase in Fe^{3+} content under adverse conditions may be due to abrupt changes in membrane permeability.

Accumulation of iron in the root apoplast has not only been found in plants grown in solution culture where Fe is generally supplied to plants as artificial siderophores but also in plants grown in soils (Masalha *et al.,* 2000) and in the roots of vine and peaches grown in the field on calcarious soils where chlorosis is common it was exceptionally high (Mengel *et al.,* 1994). According to Mass *et al.* (1988) Fe concentration in the phloem reflects the Fe nutritional status of the leaves and so may serve as a signal for Fe stress.

Owing to ferritin deposition, the Fe concentration in chloroplast may be 5 to 10 times greater than in total corresponding leaf tissue (Mengel and Bubl, 1983).

Iron enters cytosol as Ferrous (Fe^{2+}) from which may be toxic since it may be easily reacting with O_2 forming superoxide radical (Van der Mark *et al*.,1981).

In present findings, aqequate Fe content is recorded in leaf and stem tissue of *A. bracteolata* Lam. as iron is important element in oxido-reduction reactions in plants, it's performance justifies normal healthy growth of this weed in even dry arid regions.

9. Manganese

The manganese content in leaf, stem and root of *A. bracteolata* Lam. is presented in Table 11. From the Table it is evident that Manganese content eventhough in very low quantities, relatively its content is higher in stem (0.96 meq / gm) than root (0.84 mg / gm) and Leaf (0.76 mg / gm). Interestingly the highest content of Mn has been recorded in seed (1.53 mg/gm).

Usually in plants Manganese is about 5-1500 ppm of dry matter. Manganese is an important micronutrient in plants where it is involved in several enzyme systems as a cofactor especially in oxidation reduction reactions of carbohydrates and IAA. This element is involved in regulating the decarboxylating enzymes of C_4 pathways especially NADP-malic enzyme, NAD –malic enzyme and PEP carboxykinase which is highly Mn^{2+} specific. It can be replaced by magnesium generally, but also Fe, Zn and Ca.

Photosystem II is believed to contain Manganese as a prosthetic group in aprotein which catalyses the early stage of O_2 evolution (Basiounty and Biggs, 1976). It is well known that there are about 5-8 atoms of this ion per 400 molecules of chlorophyll. There is ample evidence that Mn uptake occurs in the form of Mn^{2+} presumably by facilitated diffusion across the plasmalemma (Fox and Guerinol, 1998). In a similar way to other divalent cation species, Mn^{2+} participates in cation competition. In it's chemical behavior, Mn shows properties of both alkali earth cations such as $Mg^{2+}$and $Ca^{2+}$and heavy metals (Zn, Fe). According to Wittwer and Teubenr (1959), Mn is relatively immobile in the plant and it is scarcely translocated in the phloem. This assumption is in line with results of Caballero *et al*., (1996) who found that Mn like Ca was remobilised from vegetative parts to the seeds only at the beginning of the seed formation. Lohnis (1960) found Mn concentration in *Vaccinum myrtillus* higher than 2000µ /g dry matter.

From, Electrophoretic examination of exudates it was found that Mn migrated towards the Cathode (Tiffin, 1972). Thus it appears that Mn is mainly transported as Mn^{2+} and not as an organic complex. As it's biochemical function Mn^{2+} and Mg^{2+} bridge ATP with the enzyme complex Phosphokinases and phosphotransferases. Decarboxylases and dehydrogenases of the TCA cycle are also activated by Mn^{2+}. Manganese is essential for CO_2 assimilation of C_4 and CAM plants because it activates PEP carboxylase. In Mn deficient plants the increased activity in peroxidase was probably associated with high activities of IAA oxidase (Morgan *et al.,* 1976).

The most well documented and unique role of Mn is photolysis i.e. the water splitting and O_2 evolution system in photosynthesis, the so called Hill Reaction. Proteins of the photosystem II provide most of the ligands of the Mn cluster which contains 4 Mn atoms in various oxidation states and are stabilized by Ca^{2+} and chloride (Merchant and Dreyfuss, 1998).

Because of the importance of Mn for photosynthetic electron transport, when Mn is deficient the photosynthetic light reaction seriously disturbed. In whole plants, Bussler (1958) reported tissues suffering from Mn deficiency have a small volume, cell walls dominate and the interepidermal tissue is shrunken.

From the data in Table 11 it is well evident that in *Aristolochia* Mn content is adequate in leaves to run normal photosynthesis even in arid condition during summer

period. The high Mn content in leaves are indicative of active PEPcase activity in cytosol as a part of CAM behavior reported in this weed. Its role in enzymes involved in energy transfer through ATP in Aristolochic acid synthesis will be worth to investigate. As this element shows very less mobility it's translocation is not recorded. At the same time high levels of Mn in seeds might be due to its association with aristolochic acid as seeds show strong wormicidal properties.

10. Zinc

The zinc content in leaf, stem and root of *A. bracteolata* Lam. is presented in the Table 11. It is well evident from the Table that more zinc is accumulated in roots (0.41mg / gm) while the lowest values are recorded in leaf (0.06 mg / gm). In seeds Zn content is 0.17 mg / gm dry matter.

Zn is an important micronutrient essential for plant growth and development. Zn is important for it's ability to influence auxin levels and has long been known to be a co-enzyme for production of tryptophan, a precursor for the formation of auxin (Bennett and Skoog, 2002). According to Fox and Guerinot (1998), Zn is an essential catalytic component of over 300 enzymes. It is very closely involved in the metabolism of plants. Deficiency of Zinc reduces synthesis and level of proteins.

The enzymes associated with protein synthesis catalyses the transcription of the DNA thus transfer the information of the DNA to RNA. This underlines the universal importance of Zn for fundamental processes such as the multiplication of the genetic information and the transfer of genetic information on protein synthesis. Khan *et al*., (2003) reported that Zinc application in foliar spray increased Chickpea grain yield then in control plants when well watered, but not in water stress.

Praske and Plocke (1971) have reported that Zn deficiency affected the structural integrity of the cytoplasmic ribosomes. These organelles contain high amount of Zn and in Zn deficiency they become extremely unstable.

It is well evident from the Table 11 that the Zn content in *Aristolochia* is within the range of normal and this does not indicate Zn deficiency which might have affected normal growth. It's more accumulation in leaf and stem ensures normal metabolic activity in this weed.

11. Copper

The copper content in leaf, stem, root and seeds of *A. bracteolata* Lam. is presented in the Table 11. The pattern of accumulation of copper in different parts of *A.bracteolata* Lam. is very low and as stem (0.03 mg/ gm) > leaf (0.02 mg/ gm), while in root and seed it shows minimal content (0.01mg / gm).

It is an important micronutrient essential for carbohydrate and nitrogen metabolism. Copper is required for lignin synthesis which is needed for cell wall strength and prevention of wilting. According to Epstein (1972) the optimum level of copper is 6 ppm dry wt. It also plays an important role in nitrogen metabolism, being involved in the reduction of nitrate. It is associated with enzymes involved in redox reactions of photosynthesis (Haehnel, 1984). The trace elements copper, zinc and selenium are linked together in cytosolic defense against reactive oxygen and nitrogen species. Copper, zinc–superoxide dismutase catalyzes the dismutation of superoxide to oxygen and hydrogen peroxide (Klotz *et al*., 2003). Cytochrome C oxidase , the terminal oxidase in the mitochondrial transport chain is one of the most well studied Cu containing enzyme.This unique step is of importance for all organisms generating ATP respiration. Another group of Cu proteins are the amine oxidase which catalyses oxidative deamination (Mengel and Kirby, 2006).

According to Mengel and Kirkby (1982) it is about 0.0002 to 0.002 % dry wt. Deficiency of copper induces the activity of ferric reductase enzyme, involved in Fe uptake (Kochian, 2000). The critical deficiency level of copper in vegetative parts is generally in the range of 3 to 5µg / g (0.0003-0.0005%) dry wt. depending on the plant species, plant organ, developmental stage and nitrogen supply and, this range can be larger (Robson *et al*., 1981). Whereas Mizuno and his coworkers (1982) reported that copper deficient leaves exhibited low soluble carbohydrates than normal leaves during vegetative stage.

In *Aristolochia* copper content in various plant parts follow the normal range as described for other plants. The role of Cu towards antimicrobial activity will give more idea regarding it’s role, regulation and action in this weed.

a) Organic Solutes

1. Total Free Amino Acids :

Amount of total free amino acids is presented in Table 12. It is clear that pattern of amino acid content in *Aristolochia bracteolata* Lam. is in decreasing order as leaves (38.00 mg/gm > stem (28.00 mg/gm) > roots (12.00 mg/gm). The same Table reveals that the distributions of various amino acids in different plant parts like leaf, stem and roots and are showing significant variation in its content.

Amino acids are biologically important organic compounds composed of amine (-NH_2) and carboxylic acid (-COOH) functional groups, along with a side-chain specific to each amino acid.(Wagner, 1983). Amino acids are building blocks of proteins and differ primarily in the chemical structure of their side chains, the 'R' group. Two important groups of amino acids for this set include the branched chain amino acid (leucine, valine and isoleucine) and aromatic amino acids (phenylalanine, tyrosine and tryptophan). Both these types of amino acids are produced by very tightly controlled enzymatic pathways because plants must produce all 20 essential amino acids for normal growth and development. Out of 20 amino acids Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine are essential amino acids while Tyrosine, Serine, Selenocysteine, Proline, Ornithine, Glutamine, Glycine, Cysteine, Aspartic acid, Asparagine, Alanine, Arginine, Glutamic acid are non-essential amino acids. In most of the plants, organic nitrogen is transported in the form of amino acids, which is further used for many functions including nitrogen metabolism, protein synthesis and as precursors for many important cellular constituents including nucleobases (Lalonde *et al.*, 2004). Amino acids are nitrogen source for plants in natural ecosystems and agricultural systems (Kielland *et al.*, 2006). Because of their central place in cellular Metabolism and their involvement in multiple metabolic pathways amino acids are a key focus of the developing techniques of metabolite profiling (Roessner *et al.*, 2001).

Unlike animals, plants do not derive amino acids by consuming other organisms. Therefore, all 20 of these essential amino acids must be synthesized by the plant. A challenge lies in the fact that proteins have a finite life span and must be constantly translated from m-RNA in order for plant growth and development to

Table 12: Amino acid content in different plant parts of *Aristolochia bracteolata* Lam.

Sr. No.	Plant Part	Amino acid content (µg/ gm dry tissue)
1	Leaves	3800.12 ± 0.02
2	Stem	2800.01 ± 0.01
3	Root	1200.0 ± 0.58

Data represents average of three determinations

± indicates Standard deviation

continue. This means that there must be a ready supply of all 20 amino acids for protein synthesis ultimately for plant growth and development to occur. The requirement of amino acids in essential quantities is essential to increase yield and overall quality of crops.

Plants synthesize amino acids from the primary elements, Carbon and Oxygen obtained from air, Hydrogen from water in the soil, forming Carbon Hydrates like sugars by means of photosynthesis and combining it with Nitrogen, which the plants obtain from soil, leading to synthesis of amino acids, by collateral metabolic pathways. Only L-amino acids are part of these Proteins and have metabolic activity as only L - amino acids are assimilated by plants. D - amino acids are not recognised by the enzymatic loci and therefore can not participate in protein synthesis. Glycine and Glutamic Acid are fundamental metabolites in the process of formation of vegetable tissue and chlorophyll synthesis. These amino ascids help to increase chlorophyll concentration in the plant leading to higher degree of photosynthesis. This makes crops lush Green.

Stomata are the cellular structures that control the hydric balance of the plant, the macro and micronutrient absorption and the absorption of gases. The opening of the stomatas is controlled by both external factors (light, humidity, temperature and salt concentration) and internal factors (amino acids concentration, abcisic acid etc.). L-glutamic acid acts as a cytoplasmic osmotic agent of the “guard cells”. Thus favours the opening of the stomatas.

Amino Acids are precursors or activators of phytohormones and growth substances. L - Methionine is precursor of ethylene and of growth factors such as Espermine and Espermidine, which are synsthesized from 5 - Adenosyl Methionine. L - Tryptophan is precursor for Auxin synthesis. L - Arginine induces synthesis of flower and fruit related hormones. Also L - Proline helps in fertility of Pollen. L - Lysine, L - Methionine, L - Glutamic Acid is essential amino acids for pollination. These amino acids increase the pollen germination. L - Glutamic Acid and L - Aspartic Acid, by transamination give rise to the rest of the amino acids. L - Proline & Hydroxy proline acts mainly as “osmoprotectants” on the hydric balance of the plant strengthening the cellular walls in such a way that they increase resistance to unfavourable climatic

conditions. L - Alanine, L - Valine and L - Leucine improve quality of fruits. L - Histidine helps in proper ripening of fruits (Taiz and Zeiger, 2006).

The ά-amino group of glutamate is directly involved in both the assimilation and dissimilation of ammonia and is transferred to all other amino acids. In addition, both the carbon skeleton and α -amino group form the basis for the synthesis of γ-aminobutyric acid (GABA), arginine, and proline. It should also be noted that glutamate is the precursor for chlorophyll synthesis in developing leaves (Yaronskaya *et al.,* 2006). The biochemistry and molecular biology of glutamate metabolism have been reviewed previously (Suzuki and Knaff, 2005). Aspartate is a precursor of asparagines (Lea *et al.,* 2007) and amino acids of the aspartate family, lysine, threonine, methionine, and isoleucine (Azevedo *et al.,* 2006). The germination of seeds is a period when the stored proteins are rapidly hydrolysed and transported as soluble amino acids to the growing apices of root and shoot. Furthermore, the importance of branched chain amino acid metabolism in the maintenance of tomato fruit respiration during ripening has also been recently demonstrated (Kochevenko and Fernie, 2011).

The senescence of leaves is another period when proteins are hydrolysed and amino acids are transported, again often as the amides and arginine to young developing tissue (Hortensteiner and Feller, 2002).

It is clear from our results that *Aristolochia bracteolata* Lam. leaves have more amino acids thus reflects this as major site of metabolic activity of amino acids. The dominance of this weed in hot period of summer and it's survival may be because of synthesis of free amino acids like proline against membrane impairment as osmoprotectant and enhanced proteolytic activity.

2. Total protein and Soluble Protein:

Total protein and Soluble protein content in different parts of *Aristolochia bracteolata* Lam. are recorded in Table 13 and 14 respectively. It is well evident from the Table 13 that in *Aristolochia* maximum total proteins are observed in leaf (190.28 mg/gm dry matter) followed by stem (230.68 mg/gm dry matter) and root (580.70mg/gm dry

Table 13 –Total protein content in different parts of *Aristolochia bracteolata* Lam.

Sr. No.	Plant Part	Protein content (mg /gm dry wt.)
1	Leaves	580.70
2	Stem	230.68
3	Root	190.28

Data represents average of three determinations

Table 14 –Soluble protein content in different parts of *Aristolochia bracteolata* Lam.

Sr. No.	Plant Part	Protein content (mg /gm fresh. wt.)
1	Leaves	56.01
2	Stem	28 .13
3	Root	24.00

Data represents average of three determinations

± indicates Standard deviation

matter) whereas soluble proteins (Table 14) are also highest in leaf (56.01 mg / gm) while minimum in roots (24.00 mg /gm).

Proteins are large biological molecules, or macromolecules, consisting of one or more long chains of amino acid residues. They are involved in processes such as catalyzing chemical reactions (enzymes), facilitating membrane transport, intracellular structure and energy generating reactions involving electron transport, just to name a few. Compared to animals, plants contain low levels of proteins due to the large amount of structural carbohydrate (cellulose) that compose most of a plant's structure. Proteins are constructed from building blocks called amino acids which have an amino group and a carboxyl group.

Protein synthesis and turnover in growing plants which depends ultimately upon the availability of nitrogen is basic component of metabolic regulation which provides a way for varying the enzymatic complement during response to environmental conditions (Huffakar and Peterson,1974) .

Proteins can be informally divided into three main classes, which correlate with typical tertiary structures: globular proteins, fibrous proteins, and membrane proteins. Almost all globular proteins are soluble and many are enzymes. Fibrous proteins are often structural, such as collagen, the major component of connective tissue, or keratin, the protein component of hair and nails. Membrane proteins often serve as receptors or provide channels for polar or charged molecules to pass through the cell membrane. The best-known role of proteins in the cell is as enzymes, which catalyze chemical reactions. Enzymes are usually highly specific and accelerate only one or a few chemical reactions. Enzymes carry out most of the reactions involved in metabolism, as well as manipulating DNA in processes such as DNA replication, DNA repair, and transcription. Some enzymes act on other proteins to add or remove chemical groups in a process known as post translational modification. About 4,000 reactions are known to be catalyzed by enzymes. (Bairoch, 2000). In natural conditions, soil contains a combination of organic and inorganic nitrogen forms (Chapin, 1995) and the addition of inorganic nitrogen has significantly increased the ability of *Arabidopsis* to use protein as a nitrogen source. Study shows that protein as a sole nitrogen source does not support plant growth to the

same extent as inorganic nitrogen, but that protein can supplement plant nitrogen demand (Chanyarat *et al.*, 2008).

The majority of new proteins belong to dehydrin –like proteins are abundantly induced during embryo maturation of higher plants as well as in water stressed seedlings (Pelah *et al.*, 1997).

As the leaves are the main sites of metabolic activity, it is obvious that maximum turnover of enzyme of catabolism and anabolism is recorded here. As *Aristolochia* is a dominant weed, its metabolic activities are occurring in leaf tissue and so reflecting high soluble protein content. As proteins are responsible for synthesis, transport and accumulation of different metabolites, it's distribution in different plant parts signify specific role played by them. The present findings clearly reveal that even in adverse environment, this weed thrives well because of it's undisturbed competitive metabolic strategy towards survival.

3. Total Carbohydrates

Total Carbohydrate content in different parts of *Aristolochia bracteolata* Lam. is recorded in Table 15. It is well evident from the Table that total carbohydrates are maximum in leaves (270.50 mg / gm) followed by stem (45.73 mg / gm) and finally lowest in roots (35.15 mg/gm). Same Table also depicts values of reducing sugars in leaf, stem and leaves of *Aristolochia bracteolata* Lam. and the same pattern is observed as carbohydrates.

Sugars are considered as a main source of energy provider to operate respiratory system. As early as in 1980**,** Graham showed that distorted chloroplast pigment resulted in the decrease in glucose content "first organic complex compounds" which on dimerization gives sucrose, maltose, galactose etc. and polymerization results in the formation of polysaccharides like starch, cellulose etc.

As originated from carbon dioxide and water by photosynthesis site of carbohydrates synthesis are leaves. Carbohydrates differ from the bulk of other constituents of plants in that they primarily contain carbon, hydrogen, and oxygen in 1:2:1 ratio. Some are present as phosphorylated derivatives and few others contain

Table 15 –Carbohydrate content in different parts of *Aristolochia bracteolata* Lam.

Sr. No.	Sugar	Plant Part	mg/gm fresh tissue
1	Reducing Sugars	Leaves	116 .24 ± 1.00
		Stem	113.10 ± 1.53
		Root	70.42 ± 1.15
2	Total Carbohydrate	Leaves	270.50 ± 0.57
		Stem	45.73 ± 1.12
		Root	35.15 ± 1.52

Data represents average of three determinations

± indicates Standard deviation

nitrogen. These are the principle source of energy to the plants as these are synthesized by photosynthesis, a unique mechanism found in plants. They serve as energy stores, "food" for energy supply (starch, glycogen, dextrans), production of fats, fuels, metabolic intermediates and engaged in formation of the structural framework of RNA and DNA (ribonucleic acid and deoxyribonucleic acid). They are structural elements in the cell walls of bacteria (peptidoglycans or murein), plants (cellulose and hemicelluloes), animals (chitin) and also sometimes linked to many proteins and lipids. Such linked carbohydrates i.e. glycoproteins and glycolipids are important in cell to cell communication and in interactions between cells and other elements in the cellular environment.

All living plant cells must have carbohydrates to continue life and function; however, photosynthesis occurs only in the presence of sunlight and only in cells with chloroplast. Plant cells not directly involved in photosynthesis are completely dependent on the translocation of carbohydrates from green foliage or from storage areas known as sources. Recipient tissue is referred to as a sink.

In the recent years the idea that compatible solutes might be involved in osmoregulation of higher plants has been strongly advocated (Rathert, 1983). Sugars are considered as an important metabolite in plant metabolism not only because it is the first organic compound formed in the plants as a results of photosynthesis but also provide the major source of respiratory energy. Also, sugars play a number of ecological roles in plant protection against wound, infection and detoxification of external stress (Pistocchi *et al.,* 1997). While Alaoui-Sosse *et al.,* (2004) reported that due to Cu toxicity, leaf expansion declined, leaves became a weak sink and this might account for the observed accumulation of carbohydrates in leaves. This accumulation could induce a feedback inhibition of photosynthesis. They also reported that the significant accumulation of starch and sucrose did not occur in roots and seemed to be confined to leaves. Lack of sugar utilization or formation for growth due to excessive heavy metal concentration may be stronger than inhibition of CO_2 fixation (Haider *et al.*, 2006).

In present study accumulation of reducing sugars in leaves shows active photosynthetic machinery even in arid unfavourable conditions. Also, it can be attributed towards its role as osmoticum against osmotic adjustment on dry period. The high values

of total carbohydrates in leaves are indicative of reduced transport mechanism towards the sources as roots have recorded lower values. The succulence has been observed in leaves of this weed and more accumulation of total carbohydrates especially starch may justify CAM nature of this weed under drought as this mechanism has been reported under present study.

g) Enzymes

1) Peroxidase

The activity of enzyme peroxidase in different parts like leaves, stem and root of *A. bracteolata* Lam. is recorded in Table 16. It is clear from the results that the activity of this enzyme is considerably higher in leaves (55.7 Δ O.D.h^{-1} mg^{-1} protein) as compared to stem (42.7 Δ O.D.h^{-1} mg^{-1} protein) and root (21.9 Δ O.D.h^{-1} mg^{-1} protein). As a general trend more peoxidase activity is observed in mature leaf.

Peroxidase is most widely distributed oxidative enzyme located in various subcellular components like nucleus, ribosomes, mitochondria, cell walls as membranes (Fric, 1976) whose primary reaction is to oxidize molecules at the expense of H_2O_2. Peroxidase (POX), an iron heme protein, accelerates the reduction of H_2O_2 with a concurrent oxidation of a substrate, mostly located in cell wall; it is also involved in oxidation of phenol compounds as the key enzyme for polymerization towards the synthesis of lignin (Gaspar *et al.*,1985). Peroxidase, which is generally composed of number of isozymes, is capable of catalyzing several different types of oxidative reactions including oxidation of harmones (Pilet *et al.*,1961). According to Kalir and Poljakoff-Mayber(1981) peroxidase may play an adaptive role in protecting leaf cells against oxygen toxicity caused by free radicles that may be formed in the cells when growing under saline and extreme climatic conditions.

Peroxidases from plant sources exhibit a very broad range of substrate specificity and catalyse the oxidation of cellular components such as phenolic substances, aromatic amines, ascorbic acid, ferrocytochrome C, $NADH_2$, etc. by either H_2O_2 or organic hydroperoxides (Putter, 1974).

Table 16–Activities of various oxidase enzymes in Different parts of *Aristolochia bracteolata* Lam.

Sr. No.	Enzyme Activity	Root	Stem	Leaf
1	Peroxidase (Δ O.D.h^{-1} mg^{-1} protein)	21.9 ± 0.01	42.7 ± 0.25	55.7 ± 0.29
2	Catalase (mg H_2O_2 broken down $min^{-1}mg^{-1}$ protein)	0.23 ± 0.02	0.34 ± 0.31	1.36 ± 0.02
3	IAA Oxidase (Δ O.D.h^{-1} mg^{-1})	0.04 ± 0.13	0.20 ± 0.42	0.26 ± 0.04
4	Polyphenols oxidase (Δ O. D. min^{1} mg^{1}protein)	0.57 ± 0.10	0.65 ± 0.04	0.79 ± 0.01

Data represents average of three determinations

± indicates Standard deviation

$$AH_2 + H_2O_2/(ROOH) \xrightarrow{\text{Peroxidase}} A + 2H_2O / ROH + H_2O.$$

Peroxidase also shows oxidative activity besides the peroxidation. It catalyses the oxidation of different substances by atmospheric oxygen under aerobic conditions without exogenous peroxide e.g. $NADH_2$, $NADPH_2$ (Petrochenko and Kolesnikov,1968), phenol pyruvate (Jaynes *et al.*, 1972) and Indole acetic acid (Platee *et al.* 1964). According to Mazelis and Ingraham (1962), the oxidative decarboxylation of amino acids like serine, alanine, methionine, phenylalanine and tryptophan takes place through the action of peroxidase. It also catalyzes the hydroxylation of various aromatic compounds (Paul, 1963) and reduction of NO_3^- in presence of some specific electron donar (Peive *et al.*,1972). In addition to this, peroxidases also play an important role in growth and development of plants through their control in auxin catabolism (Ray, 1962), H_2O_2 formation (Gross *et al.*,1977) and lignin as well as ethylene biosynthesis (Lieberman, 1979). According to Pilet and Gasper (1968), they may also have a function of IAA oxidase in plant cells. A number of investigations showed that the peroxidase activity in the host enhances the plant resistance (Lovrekovich *et al.* 1968).

Cell wall-located peroxidase activity has a role in cell wall formation (Ingham *et al.*, 1998). It is thought to play a key role in controlling the deposition of lignin in vascular tissue and this has been supported by the cytochemical localization of peroxidase activity in the reticulate secondary wall and adjacent primary wall of Coleus. In non lignified primary cell-walls, peroxidase is more likely to be involved in the cross linking of cinnamic acids such as ferulic acid, which are attached to cell wall polymers. Peroxidase catalyses the dehydrogenation of structurally diverse phenolic and endiolic substrates by H_2O_2 and are thus often regarded as antioxidant enzyme, protecting cells from the destructive influence of H_2O_2 and derived oxygen species (Prasad *et al.*, 1994). pH dependent cell wall peroxidases in the presence of a reductant are activated by alkaline pH, and H_2O_2 is formed (Dat *et al.*,2002). Alkalisation of the apoplast upon elicitor recognition precedes the oxidative burst and the production of H_2O_2 by pH-dependent cell wall peroxidases has been proposed as an alternative way of active oxygen species (AOS) production during biotic stress (Wojtaszek,1997).

Peroxidases participate in a great number of physiological processes, such as the biosynthesis of lignin and ethylene, defence against pathogens and wounding, auxin metabolism and stress response (Kim *et al.*,1999). Peroxidase constitutes the first line of defence against reactive oxygene species (ROS) and changes in its activity and amounts have been identified as an indicator of a redox status change under drought conditions in homoiohydric plants (Schwanz and Polle, 2001). Peroxidase enzymes participate in hormone catabolism, phenol oxidation, polysaccharides and cell wall protein intercrossing, lignin polymerization, fruit ripening, and defense against pathogens. Peroxidase has been reported to be high during early fruit growth period when most metabolic activities are at the maximum (Khali and Selselet-Attou, 2007).

High levels of peroxidise enzyme in *Aristolochia bracteolata* Lam. clearly indicate its active role in this weed and this can be attributed to effective defence mechanism in drought prone conditions. This also reflects the control measure against Reactive Oxygen Species (ROS) in this weed.

2) Catalase

Table 16 gives an idea about the performance of catalase enzyme in *A.bracteolata* Lam. Here it shows the similar pattern of decrease in the level of catalase in plant parts like leaf (1.36 mg H_2O_2 broken down $min^{-1}mg^{-1}$ protein) > stem (0.34 mg H_2O_2 broken down $min^{-1}mg^{-1}$ protein) > root (0.23 mg H_2O_2 broken down $min^{-1}mg^{-1}$ protein).

The catalase is tetrameric heme protein, occurring in almost all aerobic organisms.This enzyme is one of the few enzymes that exhibits dual enzyme activity. Plant catalases are haem enzymes that function as tetrameric proteins with four protoporphyrin IX moieties and exhibit a high molecular efficiency but a very low km that ranges between 10 and 140 mM H_2O_2 (Feierabend, 2005). Catalases are localized in peroxisomes. Hydrogen peroxide acts as a precursor of more cytotoxic or highly reactive oxygen derivatives such as peroxynitrite or OH. Thus, it is very important to scavenge excess H_2O_2 (Pang *et al.*,2005). It has hyperoxide activity (catalytic activity) when catalyses the dismutation of hydrogen peroxide into water and oxygen:

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

The other catalase activity is peroxidise activity (per-oxidative activity) when the substrates are one molecule of hydrogen peroxide and one molecule of hydrogen donar

$$\text{Donar} + H_2O_2 \longrightarrow \text{Donar oxidized} + 2\ H_2O$$

The enzyme catalase is present as multiple isoforms in plants and recent research on Catalase cDNA clone showed that catalases exist as small gene families (Scandalios, 1997). Plant catalases are predominant peroxisomal enzymes and most of them contain a carboxy terminal consensus sequence for peroxisomal import (Alberts *et al.,* 2002). It plays a role of specific peroxidase and its function is to protect cells from toxic effects of hydrogen peroxide.

Each molecule of catalase is a tetramer of four polypeptide chains. Located within this tetramer are four porphyrin heme groups that are very much like the familiar hemoglobins, cytochromes, chlorophylls and nitrogen fixing enzymes in legumes. The heme group is responsible for catalysis. Catalase found in leaf peroxisomes, functions chiefly to remove H_2O_2 formed in photorespiration or in β-oxidation of fatty acids in the glyoxysomes (Dat *et al.,* 2000). However, its activity is also found in the roots (Shalata *et al.*, 2001). It has one of the highest turnover numbers of all enzymes, one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Goodsell, 2004). Boon and his co-workers (2007) have reported that catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with hydrogen peroxide. It is an effective antioxidative enzyme in preventing oxidative damage (Mittler,2002). According to Brill (1966), catalase and peroxidase differ mutually mainly in their affinity to H_2O_2 as an electron donor. Catalase is able to catalyse even the oxidation of other substances besides H_2O_2. While, Grinberg (1971) has mentioned that since catalase has got more affinity for H_2O_2, it is mainly involved in H_2O_2 breakdown in plant tissues. Lopez-Nicolas *et al.*(2002) have demonstrated that in etiolated lupin hypocotyls, the H_2O_2 consumption by catalase was 20 to 30 times higher than guaiacol peroxidase and 200-300 times higher than ascorbate peroxidase indicating very clearly that catalase is a major H_2O_2 detoxifying enzyme. Catalase brings about H_2O_2 decomposition in the leaf peroxisomes, where H_2O_2 is generated during photorespiration by glycolate oxidase;

photorespiration rates are quite significant in C_3 plants in contrast to the C_4 species (Dat *et al.,* 2000).

Catalase catalyzes decomposition of H_2O_2 to water and oxygen, which does not need reducing substrate for its activity (Mittler, 2002). Santucci *et al.,* (2002) emphasized that catalase forms a major part of enzymatic groups of oxidoreducatases as it scavenges superoxide dismutase. Various environmental stresses (drought, chilling) have been resulted in an increase in free radicals and lipid hydroperoxides due to reduced activity of catalase (Hertwig *et al.,* 1992).

In 2009, Patil observed activity of enzyme catalase more in the leaves than that in the roots and stem in *Cassia* species. She noted that catalase activity was higher in the young leaves followed by mature leaves.

Our result are in accordance of these findings as higher Catalase activity has been reported in leaves of *Aristolochia bracteolata* Lam. As catalase is a predominant enzyme, is scavenging ROS and acting as strong antioxidant, its high activity in hot arid xeric condition is quite justifying. Also, its occurrence in roots and stem indicates the general oxidative role as oxidation of H_2O_2 generated during catalytic activity of other metabolites.

3) Indole Acetic Oxidase

It is well evident from the Table 16 that IAA oxidase activity is about seven times greater the leaves (0.26 Δ O.D.h^{-1} mg^{-1}) of *A.bracteolata* Lam. than in roots (0. 04 Δ O.D.h^{-1} mg^{-1}).

Indole acetic acid oxidase, an enzyme which catalyzes the oxidation of indole acetic acid, is almost as widespread in nature as indole acetic acid. Indole acetic acid oxidase has been found in many plants and several fungi. Levels of free IAA are, in turn, regulated via synthesis, binding, esterification, and enzymic degradation catalyzed by IAA Oxidase (Schneider,1974). One of the fungi in which this enzyme has been found is *Hygrophorous conicus.* The indole acetic acid oxidase of *Hygrophorus conicus* is an induced, intracellular enzyme (Patterson, 1965).

There have been many reports of IAAO in a wide variety of plants (Hare,1964) with the destruction capabilities increasing in a tip to base manner (Pilot, 1961) or increasing with

physiological age (Galston,1954) and inversely correlated with the IAA concentration (Kerstetter and Keitt, 1966). The physical nature of IAAO has remained controversial. Galston *et al*.,(1956) and Hare (1964) had proposed that IAAO and peroxidase activities are shown by the same enzyme protein.

The indole acetic acid oxidase system is thought to influence plant growth by regulating the concentration of endogenous IAA. Plant tissues capable of rapid growth have been found to exhibit low total IAA oxidase activity, whereas in slower growing tissues higher levels of activity was observed (Galston, 1954). However, there may be a high content of specific types of IAA oxidases in some rapidly growing cells (Lee, 1971). Direct evidence for an inverse relationship between IAA oxidase activity and endogenous IAA content has also been reported by Jain *et al*., 1969).

High IAA oxidase activity in *Aristolochia* leaves along with peroxidase suggests that enhanced protective defence mechanism. As IAA oxidase activity is considerably higher than other plants it represents slower growth of this weed. This may result in withstanding unfavourable environment and helping in its sustainance for survival.

4) Polyphenol oxidase

The activity of enzyme Polyphenol oxidase is presented in Table 16. Polyphenol oxidase activity is slightly higher in leaves of *A.bracteolata* Lam. (0.79 Δ O.D.min^{-1}mg^{-1} protein) than stem (0.65 Δ O.D.min^{-1}mg^{-1}protein) and root (0.57 Δ O.D.min^{-1}mg^{-1}protein) recorded moderately lower values. It seems that this enzyme is evenly distributed in various parts of this weed.

Polyphenol oxidase, is copper containing enzyme, also known as Catechol Oxidase, Phenolase or o-diphenol oxygen oxidoreductase, catalyses the conversion of monophenols and o-dihydroxyphenols to o-quinones in higher plants. As early as 1938, Kubowitz suggested that Catechol Oxidase is involved in aerobic respiration especially in respiratory electron transport. Sanchez *et al.,* (1997), proposed that the enzymatic browning is the main function of polyphenol oxidase in fruits and vegetables and responsible for undesirable and unpleasant sensory qualities and losses in nutrient quality of fruits. It was also indicated that cyanide insensitive respiration of plant tissues might be mediated by catechol oxidase. But the key role of alternative oxidase in this process is

now very well established. Polyphenol oxidases have been proposed to function in the Mehler reaction, photoreduction of molecular oxygen by PSI (Vaughn *et al.*, 1988).

Although, the physiological function of polyphenol oxidase in higher plants has been unequivocally determined, there is evidence that this enzyme also plays an important role in plant defense (Mayer and Harel, 1979). The plant polyphenol oxidase is induced in response to mechanical wounding and signaling molecules such as methyl jasmonate and systemin, suggesting that polyphenol oxidase may play a role in plant defense (Thipyapong and Steffens, 1997).

According to Yelena *et al.*, (1996), three types of proteins related to Polyphenol Oxidase are Catechol oxidase, Laccase and Cresolase have been identified. They catalyse two reactions: the hydroxylation of monophenols to o-diphenols (monophenolase activity) and the oxidation of o-diphenols to o- quinines (diphenolase activity). Polyphenol oxidases are important with regard to plant defense mechanism against pests and diseases and the appearance, palatability and use of plant products.

Polyphenol oxidases (PPOs) are copper containing metalloenzymes able to catalyze the oxidation of aromatic compounds by oxygen (Sanchez *et al.*, 1997). These enzymes bring about the oxidation of hydroxyphenols to their quinine derivatives, which then spontaneously polymerize. In higher plants, catechol oxidase is most prevalent form of phenol oxidase which is specific for o-hydroxylated phenols. Nuclear coded plant catechol oxidase is an intracellular enzyme located in variety of cell fractions both in organelles where it may be tightly bound to membranes and in soluble fractions of the cell. Further, the wide occurrence of this enzyme in membrane bound form in chloroplast is well documented (Mayer and Harel, 1979). Polyphenol oxidase purified from field beans (*Dolichos lablab*) which has a molecular weight of 120 ± 1-3 KDa and it is a tetramer of 30 ± 1.5 KDa, optimum pH is 4.0 and with substrates of the enzyme are 4-methyl catechol, catechol and L-3, 4 dihydroxy-phenylalanine while activity was not detected towards chlorogenic acid, catechin, caffeic acid and monophenols (Paul and Gowada, 2000). Recently, according to Hernandez-Romero (2005), there are two main types of PPOs: Laccases and Tyrosinases have been reported. Tyrosinases catalyze two kinds of reactions: ortho-hydroxylation of monophenols such as L-tyrosine, yielding L-3,

4-dihydroxyphenylalanine and the oxidation of this and other o-diphenols to o-quinones. Laccases oxidize mainly p-diphenols and methoxy substitued phenols, such as 2, 6-dimethoxyphenols.

In present studies, higher activity of PPOs in leaves of *A. bracteolata* Lam. may be for plant defense mechanism against pests and diseases. It is responsible for regulating phenolic compounds and alkaloids which are stored in all plant parts. It is concluded that it may be useful for resistance against various pathogens.

5) Nitrate Reductase

The activity of Nitrate reductase in different plant parts of *A.bracteolata* Lam. is presented in Table 17. It is clear from Table that Nitrate reductase (NR) activity is significantly higher in the leaves than root and stem thereby indicating its major role in leaves.

Nitrate reductase, a metaloflavo-protein, catalyzing the reduction of nitrate to nitrite is considered as the limiting step for conversion of nitrate–N to amino acids and so for protein synthesis and plays a primary role in assimilation of mineral nitrogen (Ferrario *et al.*, 1998). It was originally isolated from *Neurospora* by Nason and Evans (1953). Assimilation of NO_3 is a continuous process involving uptake of NO_3, its reduction to ammonia and its utilization to form amino acids and proteins. Reduction of NO_3 by plants is a fundamental biological process in which inorganic and oxidised form of N i.e. NO_3 is reduced to NH_4^+. This reduction takes place mainly in the leaves and roots. It also takes place in the embryo, cotyledons, scutella, aleurone cells etc. (Beevers and Hageman, 1969).The reduction is achieved in two stages and catalysed by two different enzyme systems, namely Nitrate reductase (NR) and Nitrite reductase (NiR).

$$NO_3^- \xrightarrow[2e^-]{NR} NO_2^- \xrightarrow[6e^-]{NiR} NH_4^+$$

Table 17– Nitrate and Nitrite reductase activities in Different parts of *Aristolochia bracteolata* Lam.

Sr. No.	Enzyme Activity	Root	Stem	Leaf
1	Nitrate reductase (µmoles NO_2 formed h^{-1} g Fresh tissue)	0.09 ± 0.13	0.12 ±0.10	0.15 ± 0.02
2	Nitrite reductase (µmoles NO_2 broken down $h^{-1}g^{-1}$ Fresh tissue)	0.13 ± 0.01	0.24 ± 0.11	0.33 ± 0.11

Data represents average of three determinations

± indicates Standard deviation

NR catalyses reduction of NO_3 to NO_2 by reduced pyridine nucleotides (NADH / NADPH, Cytochrome b 557 and Mo as the prosthetic groups. NR is a complex, oligomeric enzyme having 197 to 460 KD molecular weight. It consists of 2 subunits, one flavine protein and another molybdoflavoptotein. The enzyme possesses a mini electron transport system. It is a highly sensitive enzyme and its activity can be modified by numerous factors affecting the physiological environment of plants e.g. light, temperature, nutrients, salinity, water as well as internal factors like phytochromes and phytohormones (Srivastava, 1980)

The nitrate reduction is a rate controlling step and is regulated by activities of NR and NiR levels influenced by light, pH, temperature, CO_2 and O_2 tension, water potential, NO_3 assimilation capacity of a species and inhibitors of NR (Woodall *et al.,* 1996). According to Hewitt (1975) the synthesis of nitrate reductase is substrate regulated. There is a significant positive correlation between NR and N status of plants and their growth. NO_3 is the substrate of this inducible enzyme and also has a stabilizing effect on NR against inactivation. NO_3 translocation from root to shoot regulates supply of NO_3 to NR in the shoot parts, particularly leaves. This translocation is affected by light, carbohydrates, hormonal effects and factors like salinity which affect permeability.

Since, nitrate is the most significant source of nitrogen in crop plants, understanding the role of NR in higher plants has potential economic importance, especially in light of recent studies illuminating the enzyme as one focal point for integration of control of carbon and nitrogen metabolism (Campbell, 1999). The free glutamine level and its ratio to free glutamate, as well as the nitrate level, are probably the key metabolites governing the level of nitrate reducing capacity in a plant (Scheible *et al.,* 1997). When glutamine levels are low and nitrate is available, NR level and nitrate reducing capacity are boosted, whereas, high glutamine levels 'throttle' nitrate reduction and decrease activity levels of NR. Kaiser *et al.,* (1984) demonstrated that plant NR is probably regulated by protein phosphorylation and de-phosphorylation. According to Kabisheva *et al.* (1981), dissociation of molybdofactor from the enzyme was responsible for decrease in NR activity in salt stressed pea plants. In C_4 plants mesophyll and bundle sheath cells, exhibit difference in their NO_3 assimilation function (Marschner, 1986)

because NR is present in mesophyll cells while it is absent in bundle sheath cells (Campbell, 1999).

Pate and Atkins (1983) have postulated that a leaf located nitrate reductase may be particularly beneficial feature for plants which normally experience high levels of light, for under such conditions photoassimilation of nitrate may not have to compete directly for ATP and reducing power like $NADPH_2$ with the carbon assimilation process.

In light of this, in present findings the NR activity in *A.bracteolata* Lam. is considerably high in leaves indicating efficient nitrate metabolism and nitrogen turnover even in unfavorable conditions like hot summer. The role of this enzyme to supply N towards synthesis of aristolochic acid and other N containing secondary metabolites will be worth to investigate.

6) Nitrite Reductase (NiR) -

Table 17 depicts the activity of nitrite reductase in different parts of *A.bracteolata* Lam. Maximum activity is observed in leaf tissue (0.33 µmoles NO_2 broken down $h^{-1}g^{-1}$ Fresh tissue) while minimum in root (0.13 µmoles NO_2 broken down $h^{-1}g^{-1}$ Fresh tissue).

Nitrite, a metabolite of nitrate assimilation, is generated in the cytosol. It must be transferred immediately to the chloroplast stroma to sustain high nitrogen use efficiency and to prevent the accumulation of toxic nitrite in the cytosol (Hoff *et al.*,1994). Nitrite taken up by illuminated chloroplasts *in vitro* is studied by Brunswick and Cresswell in 1988.

Nitrite, the product of enzymatic reduction of nitrate, is further reduced to ammonia by the enzyme nitrite reductase. Nitrite reductase enzyme is present in considerably higher quantities than the nitrate reductase. From higher plants, the nitrite reduction was first demonstrated by Hageman *et al.*, (1962). The NiR is present in the chloroplast, probably in the thylakoids, while in roots it is in the proplastids. It has a marked specificity for ferredoxin as an electron donor (Vega *et al.*,1980). Nitrite the product of nitrate reduction must be further reduced to ammonia before the constituent nitrogen can enter into organic combination. This task is accomplished by a specific enzyme Nitrite Reductase, in which iron porphyrin prosthetic group, siroheme is responsible for transferring six electrons. The electrons from the reducing agents are first

accepted by iron sulphur centre, then by siroheme and lastly by nitrite, which is then reduced to NH_4^+. Light plays very important role in the regulation of this enzyme, as the reduction of NO_2^- to NH_4^+ is strictly light dependent process in green tissues.

$$\text{Nitrite} + \text{NAD(P)H} + H^+ \xrightarrow[6e^-]{\text{Nitrite reductase}} NH_4OH + H_2O + 3\text{NAD(P)}^+$$

In this reaction, there are no further intermediates and ferridoxin acts as an electron donar for enzymatic reduction (Hewitt, 1975). Now, it is well established that this enzyme is mainly located in chloroplast. At the same time, there are reports that substantial part of the activity of NiR is located outside the chloroplast also and thus there exists at least two sites for nitrite reduction in the cell (Grant *et al.,* 1970).

In chlorophyllous tissue nitrite reductase is considered to be localized within the chloroplast (Dalling *et al.,*1972) and utilises photoreduced ferredoxin flavodoxin methyl viologen to reduce nitrite. *In-vitro*, glucose-6-P has been used to reduce ferredoxin via ferredoxin-NADP reductase for reduction of nitrite (Hewitt, 1968). Further, there is some evidence that nitrite reduction may be coupled to the pentose phosphate pathway (Butt, *et al.,*1961).

It is well evident from the Table that Nitrite reductase activity is higher in leaves followed by stem and roots in this weed. As NiR activity is many folds higher than NR, it reflects the efficiency of reduction of NO_2^- to NH_4^- thereby regulating N metabolism. This *Aristolochia* belonging to Aristolochiaceae is enriched by aristolochic acid, a secondary metabolite rich in Nitrogen and the efficient N metabolism a high NR, NiR activity may be responsible for directing N to this secondary metabolite. Investigations towards biosynthesis of aristolochic acid will give more idea in this regard.

h) Photosynthesis

1. Photosynthetic Pigments –

i) Chlorophylls

Findings of seasonal photosynthetic pigment composition in *Aristolochia bracteolata* Lam. leaves are depicted in Table 18 and figure 5. It is well evident from the Table that

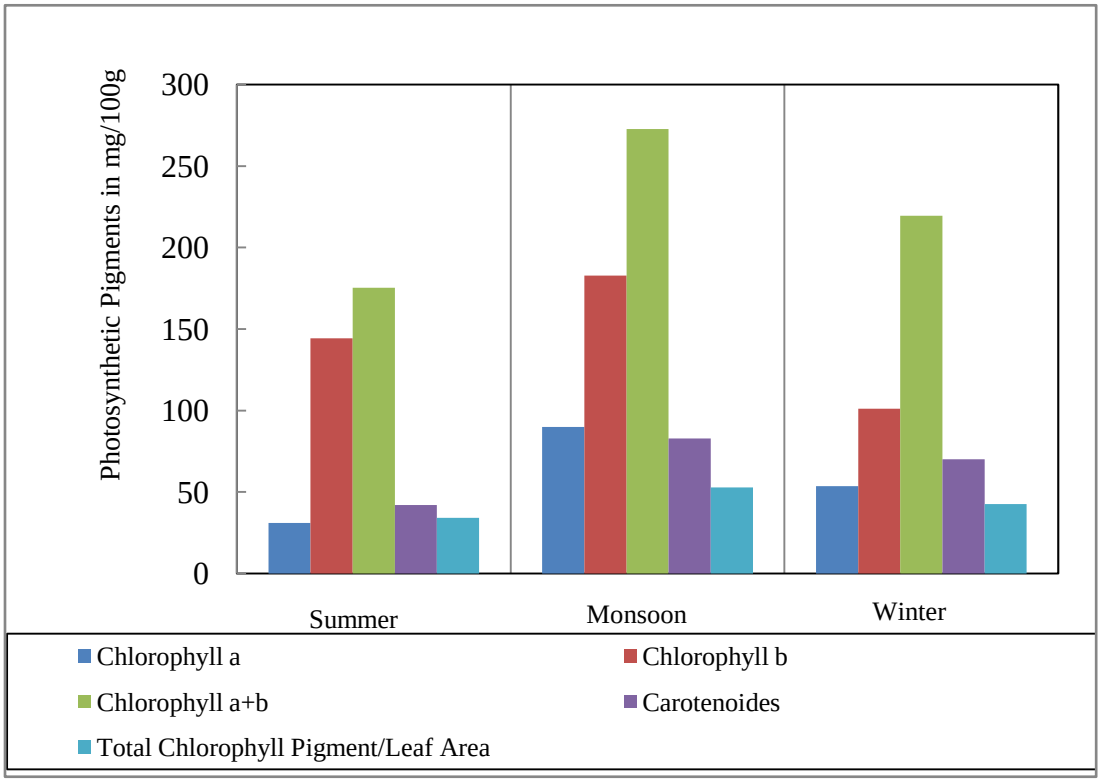
Table 18- Seasonal variations in Photosynthetic Pigments** in Leaves of *Aristolochia bracteolata* Lam.

Sr.No	Parameter	Summer	Monsoon	Winter
1	Chl a	30.93 ± 0.08	89.93 ± 0.01	53.62 ± 0.06
2	Chl b	144.4 ± 0.03	182.7 ± 0.08	101. 07 ± 0.09
3	Chl a/b	0.22 ± 0.02	0.49 ± 0.04	0.52 ± 0.07
4	Chl a+b	175.33* ± 0.61	272.63* ± 0.40	219.42* ± 0.86
5	Carotenoides	42.02 ±0.02	82.78 ± 0.03	70.08 ± 0.02
6	Chl / Carotenoides	4.17 ±0.12	3.29 ±0.14	3.14 ±0.19
7	Total chl. Pig ./leaf area	34.256* ± 0.39	52.83 * ±.053	42.52 * ± 0.54

** : mgs $100g^{-1}$ fresh tissue.

* : significant at $p < 0.05$.

Figure 5. - Seasonal Variations in Photosynthetic Pigments in Leaves of *Aristolochia bracteolata* Lam.



chl. *b* values are greater than chl. *a* in all three seasons and at the same time it is clear that total chlorophyll content is greater in monsoon than winter and summer season. It can be observed from the Table that highest total chlorophyll content (272.63 mgs $100g^{-1}$ fresh tissue) has been recorded in mansoon period followed by winter(219.42 mg/$100g^{-1}$ fresh tissue). However, total chl. content decreased in summer period (175.33 mgs $100g^{-1}$ fresh tissue), but these values are significantly high even in adverse hot conditions. This impact of unfavorable conditions is more pronounced in chl. *a* which showed about threefold decrease in the level. The impact of seasonal changes is more pronounced in chl. *a* than in chl. *b*. This is well reflected in chl. *a* / *b* ratio for all three seasons. These values of chl. *a*, chl. *b*, and total chlorophylls are statistically significant at 5% level.

Chlorophylls play a major role in light reaction of photosynthesis and hence the content as well as status of these pigments in the leaves has a direct bearing on the photosynthetic efficiency of the plant. Amongst various plant pigments chlorophylls have a unique position as these are initial binders of the solar energy. It is needless to quote endangered photosynthesis and ultimate arrested yield under adverse conditions which equally contribute to the loss of chlorophylls (Mohanty and Boyer, 1976). Chl *b* is found exclusively in the pigment antenna system, whereas Chl *a* is present in the reaction centers of photosystems I and II and in the pigment antenna. Chl. *a* is the main photosystem I pigment, which is located in exposed thyalakoid membranes, and carotenoids have the chlorophyll protection function against photoinhibition (Demmig and Adams, 1996).

Photosynthetic machinery of weed species is much competent even under unfavourable conditions and this enables them to establish successfully and compete with other plants. Sen *et al.,* (1981) have investigated chlorophyll pattern in some weeds. Not only the chlorophylls in these species exhibited consistent difference but also showed a wide range of seasonal variations during one year.

In the present findings it is well evident that a significant variation in individual chl. *a* and chl. *b* as well as total chlorophylls have been observed in three seasons and the impact of water unavailability and induced deficit affected this photosynthetic light harvesting machinery in increasing order in winter and summer. The effect of season is

more pronounced on chl. *a* than chl. *b* and this indicates that photosynthetic efficiency is lowered in summer and therefore points towards the shift in mode of photosynthesis from C_3 to CAM.

ii. Chl *a / b* ratio :

From the Table 18 and figure 4 it is well evident that Chl *a / b* ratio almost remains constant in monsoon (0.49) and winter (0.52) whereas declines drastically in Summer (0.22). The weight ratio of Chl *a* and Chl *b* (Chl *a* / *b* ratio) is an indicator of the functional pigment equipment and light adaptation of the photosynthetic apparatus (Lichtenthaler *et al.*, 1981). Also, it has been widely used an indicator of response to light intensity and senescence (Brown, 1991). Chlorophylls are particularly sensitive to oxidative attack and photodamage while carotenoids function naturally as antioxidants and in quenching photo-induced excitations.

In present findings the impact of seasonal variations is more drastic on chl.*a* than chl.*b*. In summer chl.*a* level has declined almost threefold than monsoon thereby altering chl.a / chl.b ratio. This shift is also indicative of change in the mode of photosynthesis i.e. C3 to CAM as well as sensitivity towards photooxidation.

iii. Chlorophyll / Carotenoid ratio:

The same Table (18) reveals that Chlorophyll / carotenoid ratio is highest in summer season (4.17) followed by monsoon (3.29) and winter (3.14) with minor shift.

Changes in chlorophyll / carotenoid ratio are positively sensitive indicator of oxidative damage, sensitive marker distinguishing natural full term senescence and drought and an indicator of the greenness of plants in plants (Seel *et al.*,1992). The ratio normally lies between 4.2 and 5 in sun leaves and sun-exposed plants, and between 5.5 and 7.0 in shade leaves and shade-exposed plants. Lower value for the chl /carotenoid ratio is an indicator of senescence, stress, damage to the plant and the photosynthetic apparatus, which is expressed by a faster breakdown of Chlorophylls than carotenoids (Lichtenthaler,1987). Chlorophyll loss is associated to environmental stress and the variation in total chlorophyll / carotenoid ratio may be a good indicator of stress in plants (Hendry and Price, 1993).

Sampietro *et al.*, (2007) expressed that the growth, development, dominance and allelopathic potential of any plant species mostly depends on its physiological, biochemical and enzymological characteristics. The amount of photosynthetic pigments usually correlates with photosynthetic rate, which is directly or indirectly reflected in accumulation of reserved food material like starch and dry matter accumulation rate and dry biomass.

Our values of Chl / caro ratio are lower than that of recorded for other weeds. This may be due to succulence found in this weed. The ratio is almost maintained in monsoon and and winter. However, it has risen slightly in summer and this may be because of altered mechanism of photosynthesis in summer. These findings also justify the results of pigment area ratio (Sm) as seen from the same Table.

iv. Carotenoides

Carotenoid content in *Aristolochia bracteolata* Lam. is presented in Table 18 and figure 4. Carotenoid content also followed similar pattern of chlorophylls as highest values are recorded in monsoon (82.78 mgs $100g^{-1}$ fresh tissue) and lowest in summer (42.02 mgs $100g^{-1}$ fresh tissue). Values of carotenoides are statistically significant at 5% level in all three seasons.

In addition to chl a and chl b, chloroplasts contain carotenoids as accessory pigments. Principally, carotenoid, lutein and violaxanthin have a cyclohexane ring at either end (Goodwin, 1976). Carotenoids are believed to act as supplementary light receptors broadening the range of photoreceptors and also to protect chlorophylls against photooxidation i. e. photoblenching.

Carotenoides have critical role as photoprotective compounds by quenching triplet chlorophyll and singlet oxygen derived from excess light energy, thus limiting membrane damage. This carotenoid function has been reviewed extensively by Tas and Tas (2007). Carotenoides are responsible for scavenging singlet oxygen and have many physiological functions associated with photosynthesis like structural role in the organisation of photosynthetic membrane, participation in light harvesting and energy transfer etc. (Ritz. *et al*., 2000).

Carotenoid and chlorophyll pigment content provides valuable information about the physiological status of the plant (Adams and Adams, 1992). From our results, it seems that the composition of photosynthetic pigments alongwith carotenoides in this weed is much more satisfactory to achieve maximum photosynthetic efficiency and ultimately results in maximum output in the form of high seed output as well as successful survival and establishment even under unfavourable conditions.

iv. Chlorophyll Stability Index (CSI) and Carotenoid Stability Index (CaSI):

Findings of Chlorophyll Stability Index (CSI) and Carotenoid Stability Index (CaSI) in leaves of *Aristolochia bracteolata* Lam. in different seasons are depicted in Table 19 and figure 6. It is well evident from the Table that there is marked difference in CSI and CaSI in three seasons in this weed. Highest value of Chlorophyll Stability Index is recorded in Monsoon (110.14) and lowest in summer (75.27), while high Carotenoid Stability Index is recorded in monsoon (91.73) while lowest in summer (54.04).

Earlier Sen in 1982 stated that Chlorophyll stability index is a single parameter to measure frost or drought resistance of a plant. It is defined as the difference between the colorometric readings of the chlorophyll extracts from heated and unheated leaf samples (Kaloyereas, 1958) and according to him, lower the index value higher the resistance to draught. Higher the index the lower is the resistance and vice versa

Accoring to Karyo (2006) chlorophyll degradation under stressful conditions (drought, salinity) is an effective plant adaptation, in order to reduce the flow of electrons through photosystems and to restrict the risk of photoinhibition. The alleviation of stresses can restore leaf chlorophyll content (Balaguer *et al.,* 2002). The chlorophyll stability indices during post monsoon and winter seasons were studied by Chawan *et al.,* (1979) in seven plants from Indian desert. They observed that all seven plants species exhibited a range of profiles of Chlorophyll indices and concluded that *Prosopis* sp. was most frost hardy, while *Salvadora persica* most drought resistant plant amongst the plants studied. Murumkar (1993) also studied CSI in four weeds namely *Aristolochia, Cassia, Tephrosia and Thlapsi* and observed that *A. bracteolata* Lam. showed highest CSI compared to other three weeds.

Table 19 - Seasonal variations in Chlorophyll stability index (C.S.I.) and Carotenoid Stability Index in Leaves of *Aristolochia bracteolata* Lam.

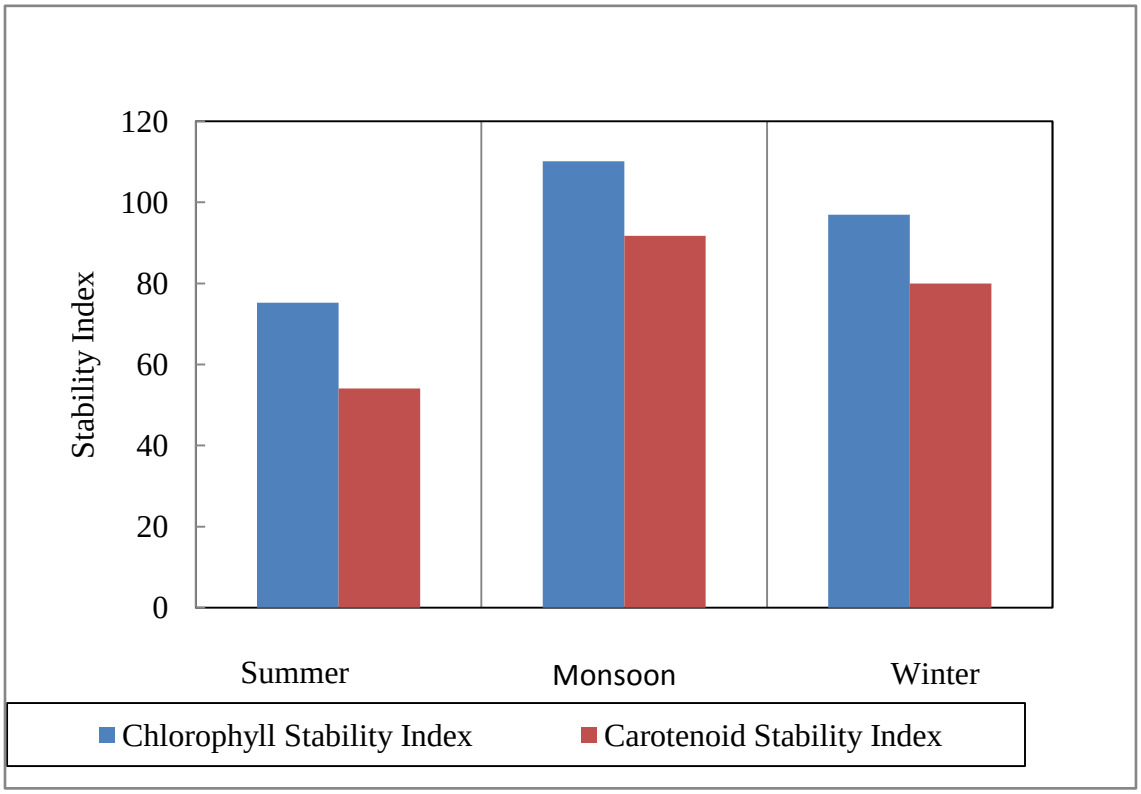
Sr.No	Parameter	Summer	Monsoon	Winter
1	Chlorophyll stability index (CSI)	75.27 *±0.46	110.14*±0.58	96.93 *± 0.81
2	Carotenoid Stability Index (CaSI)	54.04±0.55	91.73±0.25	80.00 ± 0.15

Data represents average of three determinations

± indicates Standard deviation

* : significant at $p < 0.05$.

Figure 6 -Seasonal Variations in chlorophyll stability index and Carotenoid Stability Index in Leaves of *Aristolochia bracteolata* Lam.



With above criterion, only *Cassia* exhibited true drought resistant character with lowest CSI.

As carotenoides have a protective role of chlorophylls against photooxidation, it was thought worthwhile to investigate the carotenoid Stability Index of *A.bracteolata* Lam and compare it with chlorophyll Stability Index. It can be revealed from the Table 19 and figure 5 that values of chlorophyll Stability Index are higher than the Carotenoid Stability Index. Carotenoid Stabiliy Index thus is a parameter of potentiality of the stress to damage chlorophyll molecule i.e. the unit of light harvesting molecule. A stable Carotenoid Stabiliy Index reflects the protective metabolic role towards chlorophyll stability.In *Aristolochia* high Carotenoid Stabiliy Index and succulence are playing interactive role in summer than other favourable seasons.

It is evident from the Table that chlorophyll stability index is considerably low than other two seasons thereby suggesting a higher drought resistance during summer. However low carotenoid stability index follows the same pattern and it also indicates the protective role of photooxidation of chlorophyll in adverse conditions like drought in summer.

The present findings show that the both photosynthetic pigments namely chlorophylls and carotenoides face the water stress in totally different manner and furthermore the role of carotenoides as photooxidative unit for chlorophylls is more marked in summer in this plant. This may be an adoptive feature of tolerance against drought.

Photosynthetic machinery of *Aristolochia bracteolata* Lam. has adapted itself to stabilize even under extreme drought condition as far as these indices are concerned.

2. CAM Studies

Table 20 and 21 and figure 7 and 8 are presenting Titrable Acidity Status (TAN) and pH values at seasonal variations and diurnal fluctuations in the leaves of *Aristolochia bracteolata* Lam. It was noticed that total acidity increased significantly about fourfolds during night while it lowered during the subsequent day. It is further noticed that seasonal diurnal fluctuations are also seen in pH values of leaf cell sap. Studies on the stomatal behaviour had been made and it showed that these remained open during night while

Table 20 - Diurnal flutuations in Titable Acidity Number (TAN) values in the leaves of *Aristolochia bracteolata* Lam.

Sr.No.	Time period	Summer	Monsoon	Winter
1	Morning (6 am)	226.92	125.24	47.84
2	Afternoon (12 noon)	125.89	67.60	30.50
3	Evening (6 pm)	71.93	42.72	18.71
4	Night (12 am)	123.82	66.67	35.30
5	Morning (6 am)	224.92	127.24	41.84

Data represents average of three determinations

Figure 7- Seasonal diurnal flutuations in TAN values in the leaves of *Aristolochia bracteolata* Lam.

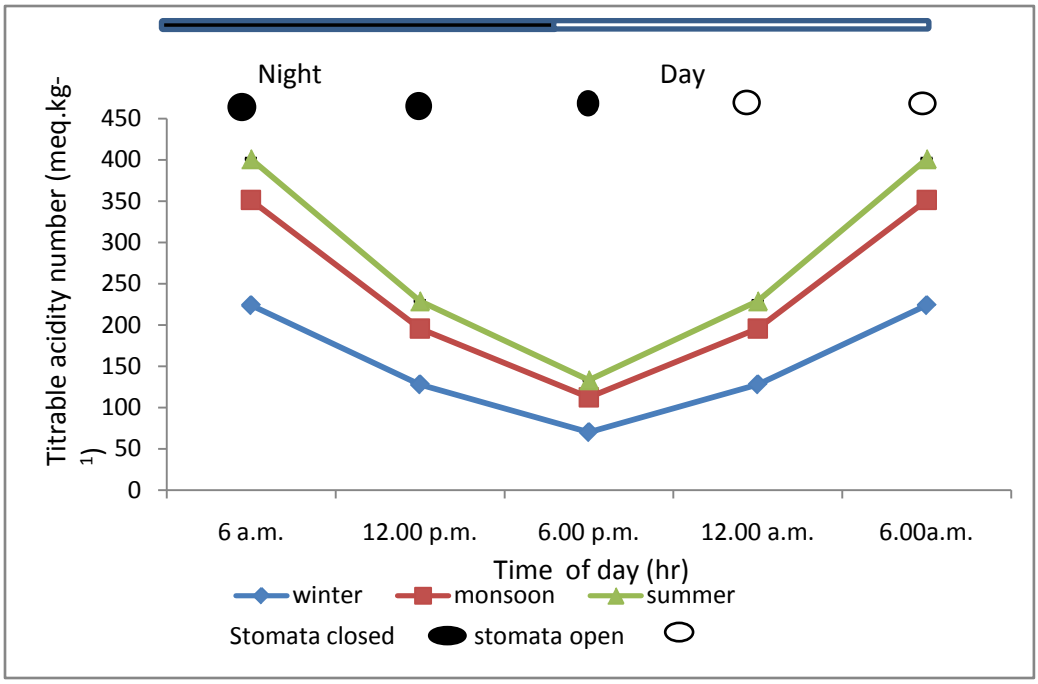
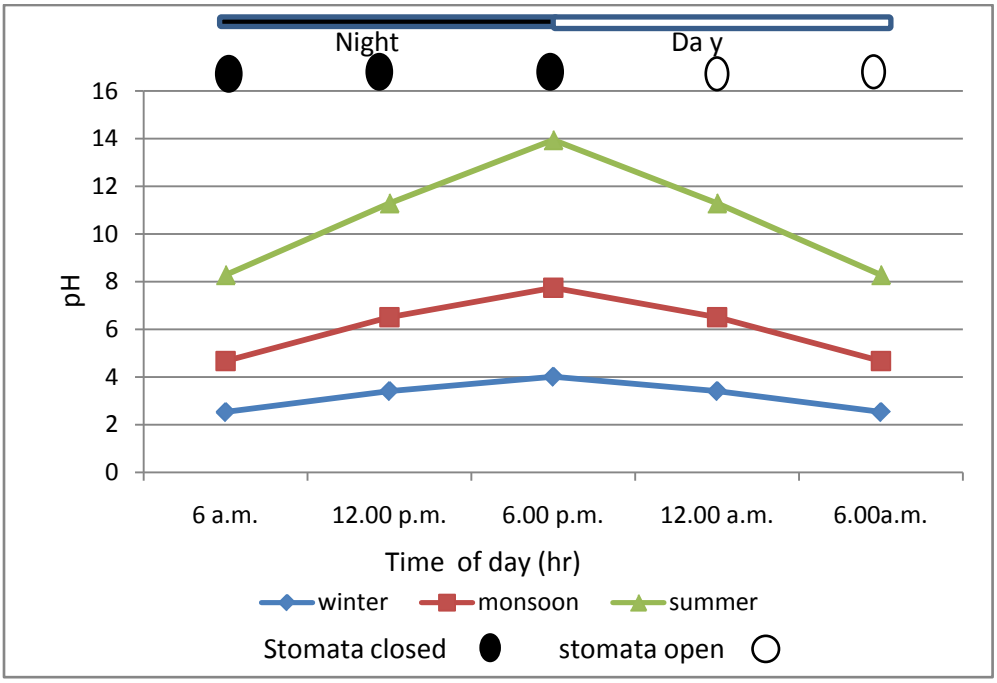


Table 21- Diurnal flutuations in pH values in the leaves of *Aristolochia bracteolata* Lam.

Sr.No.	Time period	Summer	Monsoon	Winter
1	Morning (6 am)	4.1	2.3	2.5
2	Afternoon (12 noon)	5.4	3.2	3.4
3	Evening (6 pm)	6.2	4.5	4.2
4	Night (12 am)	5.2	3.4	3.3
5	Morning (6 am)	4.2	2.2	2.7

Data represents average of three determinations

Figure 8 Seasonal diurnal flutuations in pH values in the leaves of *Aristolochia bracteolata* Lam



closed during day time. All these findings strongly advocated that CAM mode of photosynthesis prevails in this weed.

In extreme habitats, plants exhibit versatile photosynthetic pathways in response to environmental variables such as temperature and moisture. One such variation of carbon acquisition, Crassulacean Acid Metabolism (CAM), is often employed by plants in arid regions, where they usually face a "desiccation-starvation dilemma" because they have to acquire enough carbon to survive while minimizing the amount of water loss through stomatal activities and other physiological and biochemical functions.

Crassulacean Acid Metabolism (CAM) is a modified photosynthetic adaptation that temporally separates the C_4 pathway and the Calvin Cycle by time, and plants that utilize this type of photosynthesis fix CO_2 during night time. The stomata are closed during the day, allowing increased water conservation and CO_2 concentration thus retarding water loss, and open during the night. This action results in a low transpiration rate and high water-use efficiency.

CAM photosynthesis is adapted by a large number of vascular plants in arid regions for water conservation and CO_2 concentration. The nocturnal CO_2 fixation is done by the cytosolic enzyme phosphoenolpyruvate carboxylase (PEPC). The CO_2 is fixed into malate, and stored in the vacuole within the same cell as malic acid- an organic acid that serves as a CO_2 reservoir at night and during day time CO_2 re-assimilated by the normal C_3 (Calvin Cycle) photosynthetic pathway (Luttge, 2004). Decker and de wit in 2006, postulated that CAM plants are also succulents and form a significant component of tropical and subtropical environment of arid climate. These plants are relatively rare globally, as they represent a significant fraction of regulation mechanism in some hot, arid environments, perhaps evolved from early hot arid conditions as early as Triassic. CAM plants can effectively save metabolic energy and water during harsh environmental conditions by closing their stomata during the day (Taiz and Zeiger 2010).

According to Szarek, (1979) plants which are categorised as CAM group as opposed to the C_3 Calvin cycle pathway or the C_4 dicarboxylic acid pathway, are distinguished from latter groups because: i) a massive circadian variation occurs in titraTable acidity of chlorophyllous tissue (ii) Stomatal opening and the carboxylation of

exogenous CO_2 are nonautotrophic processes (iii) high δ^{13} values, i.e. -10 to -18 %, indicate the coupling of CO_2 assimilation and malic acid accumulation, and (iv) there is long temporal separation of net CO_2 assimilation and net carbohydrate (starch) accumulation. Other criteria have been used for identifying the potential presence of CAM, e.g succulence of photosynthetic tissue, but these may be less diagnostic than the physiological tissue.

Feakins (2010) proposed that CAM plants are more enriched by NADPH for lipid biosynthesis is generated more by heterotrophic pathways (probably the PPP) than by photosynthesis. This would be consistent with their slower growth rate during CAM metabolism. Study on Sampling 20 species of plants grown in greenhouse conditions, demonstrated that CAM-enabled species had a wide range of carbon isotopic compositions in both leaf wax lipids and bulk tissue. A positive correlation between δ 13C bulk and δ D_{C31} values implicated a metabolic isotope effect as the dominant cause of interspecies variation in the hydrogen isotopic composition of leaf wax n-alkanes in CAM-intermediate plants.

In an earlier survey, as many as 3 divisions, 26 families, 159 genera and 612 species have been reported to possess CAM (Szarek, 1979). To date, approximately 7% of plant species, encompassing 33 families and 328 genera are known to possess CAM (Winter and Smith, 1996). Such taxonomic diversity is reflected by the range of habitats favoured for CAM plants which range from arid deserts, tropical rainforests to aquatic ecosystems. Most notable among these are commercially or horticultural important plants such as pineapple (*Ananas comosus*), Agave (*Agave* subsp.), Cacti (Cactaceae) and orchids (Orchidaceae). Thus, CAM is typically, although not exclusively, associated with plants that inhabit extremely arid environments (e.g. deserts), semi-arid regions with seasonal water availability (e.g. Mediterranean climates), or habitats with intermittent water supply (e.g. tropical epiphytic habitats).

CAM plants are found at contrasting sites in many different ecosystems and are highly polyphyletic with approximately 16000 species and facultative CAM plants commonly exist in places with seasonal climate fluctuations, and unpredictable periods of stress (Winter, 1985). Guralnick and Ting, (1987) classified CAM plants into two categories obligate and facultative CAM and proposed that obligate types exhibit CAM

pathway under all conditions whereas facultative types show C_3 pathway under well watered conditions but they shift towards CAM when water becomes less available. Whereas according to Luttge, (2004) facultative CAM plants are highly flexible in their method of carbon acquisition in that they utilize the C_3 pathway during favourable conditions such as mild temperature and abundant precipitation, but switch to a CAM mode when stressed (e.g. drought). The level of expression from C_3 to CAM in plants is on a continum, with C_3 and CAM being the extremes. Once CAM is induced, it is highly reversible depending on various changing environmental conditions (Lee and Griffiths, 1987). And this C_3-CAM switch is important in maintaining viable physiological performance under stress; thus, facultative CAM plants are very sensitive to changes in environmental factors (Winter, 1996).

Recently, induction of Crassulacean Acid Metabolism in the facultative halophyte *Mesembryanthemum crystallinum* by abscisic acid was studied by Chun *et al.*(1990). They demonstrated that exogenously applied ABA can substitute for salinity or drought in inducing the expression of CAM in *M. crystallinum,* and this induction is dependent on development as in young plants, neither salt nor ABA can induce CAM. This suggests that some factors, in addition to ABA, are required for induction. Similarly Facultative CAM is reported in weed *Thlaspi arvence* L. by Murumkar *et al.,* (1991), in *Aristolochia bracteata* Retz. (Deshmukh and Murumkar, 1996), *Commelina nudiflora* (Deshmukh and Murumkar, 2013).

Analysis of approximately 40 genes in the inducible CAM plant, *Mesembryanthemum crystallinum* have indicated rhythmic changes in transcript abundance of more than 30 of the selected genes with expression peaks at various phases throughout the diurnal cycle. Moreover, more genes show rhythmic changes in transcript abundance in plants expressing CAM compared with plants in the C_3 state (Boxall *et al.*,2002). Mechanisms exist for `sensing' carbohydrate availability throughout the night since the shift from $C_3$ to CAM results in sustained `metering' of carbohydrate reserves, which maintains substrate availability for the duration of the dark period. One way in which the clock could synchronize the metabolic components of CAM would be to phase the transcription of particular genes to specific times in the day/night cycle, thus ensuring that appropriate enzymes and transporters are most abundant when required. Genes found

to be controlled by the clock in CAM-performing *M. crystallinum* encode enzymes involved in photosynthesis, glycolysis, nocturnal CO_2 uptake, decarboxylation, sucrose and starch metabolism (Dodd *et al.*, 2003). The gene that encodes PPCK, the protein responsible for activating PEPC, is expressed at comparable levels in leaves of both C_3 and CAM-performing species of *Clusia* and day / night changes in the expression of PPCK appear to be a consequence of the diel cycling of organic acids and soluble sugars (Taybi *et al.*, 2004).

A key role for chloroplast transporters in regulating carbohydrate turnover over the diel cycle is indicated by the finding that chloroplasts of CAM-performing leaves of *M. crystallinum* are unique in containing three classes of phosphate translocators (i.e. PEP, triose-P, and glucose-6-P translocators), in addition to a chloroplast glucose transporter (Haeusler *et al.*,2000). CAM is also correlated with various anatomical or morphological alterations that minimize water loss, including thick cuticles, low surface-to-volume ratios, large cells and vacuoles with enhanced water storage capacity (i.e. succulence), and reduced stomatal size and/or frequency.

Intensive ecophysiological studies over the last 20 years have documented that CAM is present in approximately 7% of vascular plant species, a much larger percentage than the percentage of C_4 species (Winter and Smith, 1996). The widespread distribution of CAM among 33 taxonomically diverse families suggests that CAM most likely evolved independently on numerous occasions in different families and even within individual families. It is curious that CAM is also found in aquatic vascular plants where it presumably enhances inorganic carbon acquisition in certain aquatic environments where CO_2 availability can become rate limiting for photosynthesis (Keeley, 1998). Thus, the daytime limitation of CO_2 availability brought about by water-conserving stomatal closure in arid terrestrial habitats or by competition from other species and the high diffusional resistances limiting access to CO_2 in aquatic habitats, appears to be the common factor responsible for the evolution of CAM.

Drennan and Nobel, (2000) concluded that many families bearing CAM belong to tropic and subtropics. The net effects of this CO_2 concentration strategy is that CAM plants exhibit water use efficiency (WUE) rates several fold higher than C_3 and C_4 plants under compatible conditions whereas according to Sage (2002) an initial step in CAM

evolution is thought to encompass the nocturnal scavenging of respiratory CO_2 by PEPC, a feature that was probably facilitated by the succulent and large vacuolated leaf anatomy that is typical of CAM species and succulence may have been a pre-existing trait that improved hydraulic transport and capacitance in habitats with high transpiration demand. Since CAM is usually considered to have arisen in extreme environments it is conceivable that the progenitors of CAM already possessed clocks with a pervasive control over metabolism as a means of maintaining homeostasis in potentially limiting environments (Raven and Spicer, 1996).

In present investigation, we have observed that there is nocturnal CO_2 uptake through open stomata at night. There is more than 3-4 fold increase in malic acid content at night in all seasons and also significant diurnal fluctuations in titrable acidity status and pH values. Leaves also show high degree of succulence. All these findings strongly advocate that in *Aristolochia bracteolata* Lam. facultative CAM mode of photosynthesis prevails because this effect is more profound and significant in unfavourable hot summer season than favourable rainy and winter period.

A report on CAM behaviour of *A. bracteata* Lam. is signifying the response of this plant in arid region. But occurrence of this weed throughout the year leads to the speculation that this plant was exhibiting facultative CAM. The findings are confirming our hypothesis that CAM behaviour in this weed is of adoptive response towards the environment and also justifies the facultative CAM nature.

3. Hill Reaction:

The activity of Hill Reaction is presented in Table 22 and figure 10 It is well evident from the Table that activity of Hill reaction in isolated chloroplast show strong progressive reduction of DCPIP after 10 minutes and about 13 % reduction of DCPIP has been observed after 20 minutes.

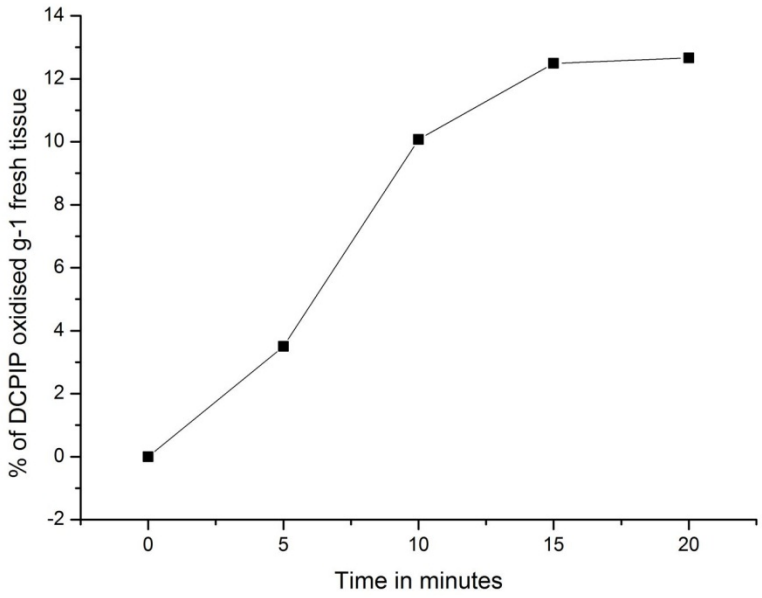
Earlier in 1937, Hill discovered that isolated chloroplasts can generate oxygen when they are illuminated in the presence of a suitable electron acceptor, even if no

Table 22- Hill Reaction demonstrated in Leaves of *Aristolochia bracteolata* Lam.

Sr.No	Time (Min)	% DCPIP oxidised g^{-1} fresh tissue
1	0	0.00
2	5	3.50
3	10	10.07
4	15	12.49
5	20	12.66

Data represents average of three determinations

Figure 9: Hill Reaction demonstrated in Leaves of *Aristolochia bracteolata Lam*.



carbon dioxide is present. Plant Productivity is associated with the process of photosynthesis. It is essentially an oxido-reduction process in which photolysis of water (Hill reaction) takes place with the help of chlorophyll and light and the electrons generated are used in the formation of reducing power $NADPH_2$, which is finally utilized in the reduction of CO_2 to carbohydrate level. The chemical reduction by which water is oxidized to a pair of protons and four electrons is achieved by photolysis of water or Hill Reaction. Here, two water molecules are involved in generating four electrons and a molecule of oxygen. This oxidation is achieved by an electron acceptor like Ferredoxin. In recent experiments this has been demonstrated by using DCPIP dye. In present work, Hill activity was measured using DCPIP and from the findings it is quite clear that even under drought conditions and at high temperature Hill reaction is not showing any potential inhibition in the action indicating the undisturbed PSII in this plant.

Early report of Hill reaction in *Nostoc muscorum* and *Chlorella pyrenoidosa* Brown (1954) *and* in *Chlorella pyrenoidosa* by Eyster *et al.* (1956) with respect to role manganese for the Hill reaction is well documented.

Effect of pollution on Hill reaction has been studied by many workers. Behara and Mishra (1983) studied effect of industrial effluents on Hill reaction of rice seedlings and concluded that loss in Hill reaction activity in isolated chloroplasts could be correlated in a general way with the loss of pigments, proteins and nucleic acids. Whereas Mandal and Mukherji (2000) studied changes in chlorophyll context, chlorophyllase activity, Hill reaction, photosynthetic CO_2 uptake, sugar and starch contents in five dicotyledonous plants exposed to automobile exhaust pollution and concluded that chlorophyll content of dust polluted leaves was lower than that of control leaves

Murumkar in 2001 studied the Hill reaction in *Cassia auriculata* L. leaves and showed strong progressive reduction upto 70 % after 20 minutes.

From the reduction percentage in Hill reaction in *Aristolochia bracteolata* Lam. leaves, it is clear that the slow rate of photolysis can be attributed to rate limiting photosynthesis alongwith nitrogen metabolism as this plant is diverting the carbon more towards synthesis of secondary metabolite Aristolochic acid.

4. Photo-oxidation of Chlorophyll Molecules :

The effect of oxidation of photosynthetic pigments *chl* a, *chl*.b, total chlorophylls (Chl. a+ b) and carotenoides is presented in Table 23 and figure 9. It is well evident from the Table that treatments like exposure to light, dark and heat have marked varying effect on these pigments.

The effect of photo-oxidation on photosynthetic pigments is more profound on *chl* b than *chl* a, with overall drastic decline in these two pigments. Incubation in dark also showed similar trend with *chl* b oxidizing at slower rate while chl.a content showing slight increment indicating diversion of carbon skeleton towards synthesis of *chl* a in dark incubation. Effect of heat treatment shows that *chl* a is more susceptible than *chl* b indicating unsTable bonding in *chl* a than *chl* b.

The same Table revealed that with time, light as well as heat has triggered overall oxidation of these photosynthetic pigments (total chlorophylls) and this effect is more severe in photo-oxidation than other treatments.

First credit goes to extensive study of photo-oxidation of chlorophyll using esteroscopy. to Tollin and his co workers (1962). They observed that chlorophylls were able to produce steady state signals of semiquinone anion radicles by irradiating alcoholic solution of chlorophyll containing various quinines.

Arnoff and Mackinney in 1943 exposed chlorophyll in acetone to light in presence of oxygen. It was irreversly bleached and pink intermediates were formed. Quite stable if kept in dark for several days and exhibited orange fluorescence on continued exposure the solution becames colourless and devoid of fluorescence.

Early in 1970, Kessler studied photo-oxidation of chlorophyll molecules in normal and manganese-deficient, closely related *Chlorella* strains with hydrogenase *Chlorella vulgaris* f. *tertia* 211-8k) and without hydrogenase *Chlorella vulgaris* 211-8m. He observed that Photooxidation of chlorophyll under pure O_2 in very strong light is always accelerated by Mn deficiency and presence of hydrogenase may protect chlorophyll against destructiion when cells are Mn deficient.

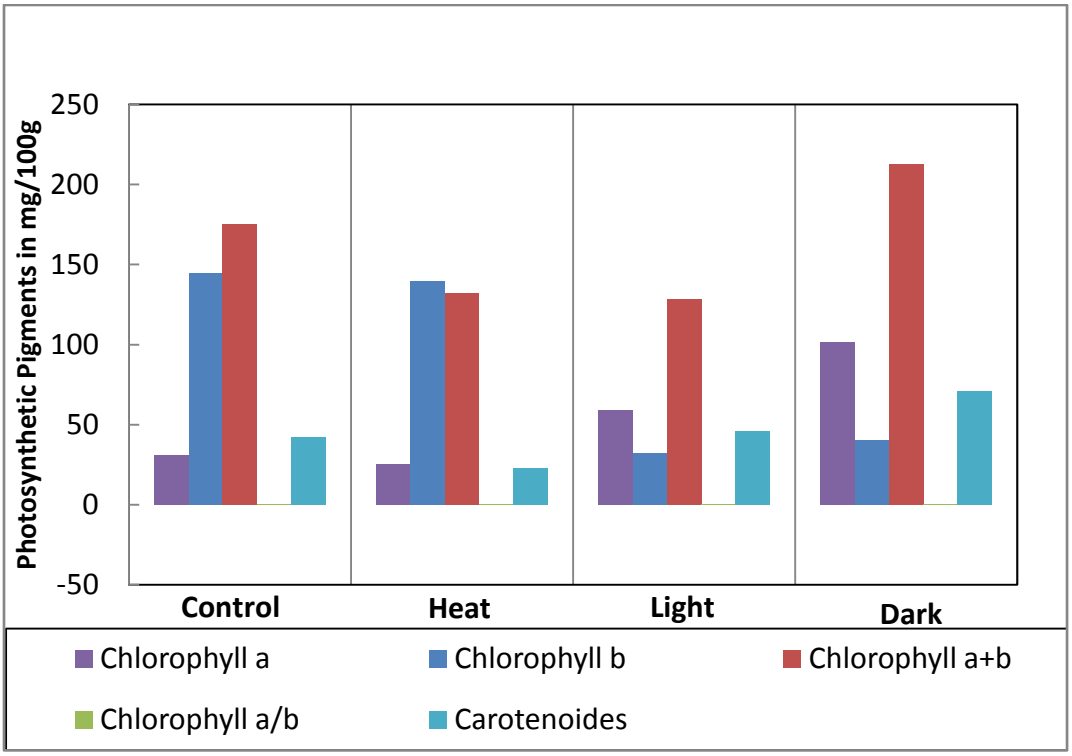
Table 23 –Effect of different treatments on oxidation of Photosynthetic pigments** in leaves of *Aristolochia bracteolata* Lam.

Sr. No	Parameter	Control	Heat Treatment	Light	Dark
1	Chl a	30.93 ± 0.58	25.64 ± 0.02*	32.2 ± 0.01*	40.58 ± 0.01*
2	Chl b	144.4 ± 0.73	139.9 ± 0.44*	59.00 ±0.01*	101.5±0.02*
3	Chl a+b	175.33 ±0.03	165.34 ±0.02*	91.2 ±0.02	142.08 ± 0.50
4	Chl a/b	0.21 ±0.002	0.18 ± 0.001*	0.55±0.002*	0.40 ± 0.01*
5	Carotenoides	42.00 ± 0.01	22.7 ± 0.1*	46.1 ± 0.02*	70.8 ± 0.1*

**: mgs $100g^{-1}$ fresh tissue.

* : significant at $p < 0.05$ when compared to control

Figure 10 –Photo-oxidation of Photosynthetic pigments** in leaves of *Aristolochia bracteolata* Lam.



Tomkiewicz and Klein (1973) worked on Photo-oxidation of Chlorophyll b studied by Chemically-Induced Dynamic Nuclear Polarization and reported the direct evidence of the nature of the precursors in the photooxidation of chlorophyll is by quinones. Drought affects Photosystem II more than Photosystem I in the photosynthetic mechanism. They become uncoupled, resulting in free, high-energy electrons in the leaf. Uncoupled electron transport leads to photo-oxidation of chlorophyll and loss of photosynthetic capacity. A very obvious bleaching of leaves exposed directly to the sun under drought stress can be observed (Banziger *et al.*,2000).

These findings and above discussion clearly indicate stimulated photoblenching in detached pigments whereas detioration of these pigments under heat and dark is much slower as compared to light..

i) Secondary metabolites

1) Free Proline

The free proline content in leaf, stem and root of *Aristolochia bracteolata* Lam. is presented in Table 24. Highest proline content is recorded in leaf (3.32 mg/g) followed by stem (1.85 mg/g) and lowest in root (1.10 mg/g). Thus, in this plant leaf showed three times high proline content in shoot part as well as in root indicating it's active role and action.

Aspinall and Paleg, (1981) stated that proline is an important secondary cyclic amino acid belonging to glutamic acid family and dramatic increase in free proline content has been reported in many plants subjected to various environmental stresses such as drought, salt and cold. It protects membranes and proteins against the adverse effects of high concentrations of inorganic ions and temperature extremes (Santoro *et al.*, 1992), and functions as a protein-compatible hydrotrope (Srinivas and Balasubramanian, 1995), a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989), utilizable source of energy Singh *et al.,* (1973). Proline is osmotically very active, contributes to membrane stability and mitigates the effect of NaCl on cell membrane disruption (Mansour, 1998).

Table 24– Free Proline content in different parts of *Aristolochia bracteolata* Lam.

Sr.No.	Plant Part	Proline content (mgs g^{-1} Fr. Tissue)
1	Leaves	3.320 ±0.020
2	Stem	1.850 ± 0.001
3	Root	1.100 ± 0.002

Data represents average of three determinations

± indicates Standard deviation

Proline, which occurs widely in higher plants, accumulates in larger amounts than other amino acids (Abraham *et al*., 2003) and regulates the accumulation of usable proline in cytosol where it contributes substantially to the cytoplasmic osmotic adjustment (Ketchum *et al*., 1991).

Kavi- Kishor *et al.*, (2005) proposed that it has diverse functions as stabilization of sub cellular structures of Proteins and membranes, as hydroxyls radical scavenges and serve as source of Carbon and Nitrogen. In addition, Proline can act as a component of signal transduction pathway that regulates stress responsive. Adjusting nitrogen transport in response to environmental perturbations is very important and such regulation has been observed in the case of the proline transporter *AtProT2*, which is strongly induced under conditions of water stress and salt stress (Rentisch *et al.,* 1996). Flower and Ludlow (1986) had proposed that solute like proline accumulates in meristimatic or any other similar tissue in a severally dehydrated plants and it may be maintaining cell volume, metabolic concentrations, maintaining turgur that is to keep the volume above a critical value and this could help for faster recovery of the plant, when the soil is rewatered.

Maggio and his co workers (2002) concluded that proline may act as a signaling regulatory molecule, activate multiple responses for adaptation process. Earlier, Schobert (1977) suggested that the accumulation of proline and glycine-betaine in plant tissue should not be seen as a cytoplasmic osmoregularotary process but as non-osmotically linked protective phenomenon maintaining hydration of macromolecules possibly by directly proline - protein association.

Extensive work has been carried out which focuses on the important role played by this amino acid in stress resistance. The best characterised biochemical response of plant cells to osmotic stress is the accumulation of organic osmolite, most commonly proline and betaines. To counteract the effects of osmotic stress, plants have evolved adaptive mechanisms which may be classified into four categories. Three of these adaptations: developmental traits (e. g. time of flowering), structural traits (e.g. leaf waxiness) and physiological mechanisms (e.g. the ability to exclude salt while maintaining the absorption of water and ability to compartmental ions within the vacuole), involve complex interactions. The gene products controlling these traits have

remained largely uncharacterised. The fourth category involves metabolic responses such as alteration in photosynthetic metabolism and the accumulation of compatible osmolytes. The later could be due to a small number of special gene products which may be identified as osmotically induced changes in protein and mRNA population (Mc Cue and Hansson, 1990). The absence of positive correlation between proline accumulation and osmo -tolerance in some species does not negate an adaptive role for proline rather, it may reflect the predominance in these species of osmoregulatory mechanisms other than osmotic adjustment (Caplon *et al.*,1990). This statement is confimed by the work of Erakar and Murumkar (1995) in weed *Tephrosia purpurea* Pers. where they observed that higher proline content in root tissue followed by leaf and stem.

According to Stewart (1981) equilibrium exists between proline synthesis and degradation, and hence no significant promotion in proline accumulation is evident in non-stressed as well as mildly stressed plants. On the contrary, in stressed plants proline degradation is relatively lowered down while proline synthesis is greatly promoted resulting in proline accumulation. Proline synthesis is implicated as a mechanism of alleviating cytoplasmic acidosis, and may maintain $NADP^+$/NADPH ratios at values compatible with metabolism (Hare and Cress, 1997).

There are two alternative routes in proline biosynthesis in higher plants: as the L-arnithine and the L-glutamate are two precursors of proline biosynthesis. Under osmotic stress, the glutamate pathway is the main source of proline production. Proline oxidase is the main regulatory enzyme responsible for accumulation of osmolytes, it converts proline to glutamate (Adams and Franks,1980).

As, *Aristolochia bracteolata* Lam. is a weed from dry arid region, accumulation of proline in different parts may be towards osmotic adjustment of this plant to avoid environmental stress and more amount in leaf tissue probably justifies its role as an osmoticum towards cell protection maintaining hydration of macromolecules by proline association.

2) Total Polyphenols

Table 25 represents distribution of total polyphenols in vegetative parts of *Aristolochia bracteolata* Lam. It is evident from the Table that polyphenol its content is greater in leaves (15.20 mg/g) while root (4.40 mg/g) and Stem (4.61mg /g) shows marginal polyphenol content.

Secondary metabolic products containing a phenol group a hydroxyl functional group on an aromatic ring are designated as phenolic compounds. These form a chemically heterogeneous group of nearly 10,000 individual compounds. Polyphenolic compounds are present in most of the phanerogamic species. Included within this group of compounds are phenolic acids, flavonoids and other related substances with an unsophisticated structure. Some phenols are soluble only in organic solvents while others are water soluble carboxylic acids and glycosides and others are large, insoluble polymers (Taiz and Zeiger, 2006)

Polyphenols represent the products of secondary metabolism. Plants produce a large, diverse array of organic compounds that do not appear to have any direct function in growth and development. Plant secondary products can be divided into three groups according to their mode of biosynthesis - terpenes, phenolics and nitrogen containing compounds (alkaloides). Of these, phenolic compounds are aromatic sunstances formed via shikimic acid pathway or malonic acid pathway in various ways. Plant phenolics are chemically heterogeneous group, some are soluble only in organic solvent, and some are water soluble carboxylic acids and glycosides, while others are insoluble large polymers. Polyphenols are organic compounds having more than one OH group. They are formed during secondary metabolism. They include substances from catechol having 2 OH groups, various tannins, hydroxyl cinnamic acid, flavonoid pigments, lignin, melanin, betallins etc. which are widely distributed in plants. Anthicyanins, leucoanthocyanins, anthoxanthine, hydroxybenzoic acid, glucosides, and sugar esters of quinine derivatives make a big class of phenolics. The functions of polyphenols are variable e.g. lignin reduces transpiration, anthocyanins give colour to flowers. They interfere with grown and energy requiring metabolism by uncoupling oxidative phosphorylayion. On oxidation, phenols yield quinines, which form a complex with metal ion reacting with sulphahydral

Table 25: Total Polyphenol content in different plant parts of *Aristolochia bracteolata* Lam.

Sr. No.	Plant Part	Total Polyphenol content (mg/g fresh tissue)
1	Leaves	15.20±0.03
2	Stem	4.61± 0.02
3	Root	4.40 ±0.01

Data represents average of three determinations

± indicates Standard deviation

group or binding nonspecifically to enzyme protein. As a result, enzyme activity is inhibited. Hence, small changes in phenol level may affect physiological processes like photosynthesis, respiration, chlorophyll synthesis, membrane permeability, enzyme synthesis and it's actions etc. There are different external factors responsible for changes in the level and comparison of polyphenols. Strognov (1964) suggested that stimulation of secondary metabolism due to salinity results in increase in polyphenol content of plants.

In keeping record with their chemical diversity, phenolics play variety of roles in the plant. Many have a role in defence against herbivores and plant pathogens. Others function in mechanical support, in attracting pollinators and fruit dispersers or in reducing the growth of nearby competing plants. (Goodman *et al.*,1964). The role of polyphenols in plant metabolism is of interest for several reasons. Their wide spread occurrence in higher plants suggest a possible significant induction in the metabolism of the plant.

According to Faruya *et al.*,(1962) flavonoides may affect plant growth by controlling the activities of IAA oxidase system. The action of phenolic compounds is not related to their structural configuration.

This is quite natural that the synthesis of phenolic compounds is influenced by several environmental and endogenous factors which are bound to change during life cycle of the plant. Polyphenol synthesis has been found to be regulated by light (Zucher *et al.*,1965).

In 1993, Murumkar in his work on ecophysiological studies in weed species of Baramati region studied total polyphenol content in four weeds namely *Thlaspi arvense, Tephrosia purpurea, Aristolochia bracteolata, Cassia auriculata.* He observed higher polyphenol content in *Aristolochia bracteolata*. Similarly, in 2001, he studied polyphenol content in a xeric weed *Cassia auriculata* Lam. and reported that polyphenols accumulated in bark tissue followed by leaf and these values are higher than the values recorded for other plants (Sen, 1982).

Total ploy phenol content in *Aristolochia bracteolata* Lam. leaves shows higher levels, which may be acting as defense mechanism against herbivores. Due to its bitter

taste and herbaceous nature when this weed is exposed to open grazing field the weed may get protected and thus, avoided by herbivores.

3) Tannins

In Table 26, the distribution of tannins in *Aristolochia bracteolata* Lam. has been presented. It is observed that more amount of tannin is accumulated in leaves (1.48 mg/g) while lowest value is recorded in root tissue (0.50 mg/g). Its accumulation in leaf is almost three times higher than roots.

One of the major phenolic polymers with defensive properties is tannins. The term tannin was first used to describe compounds that could convert raw animal hides into leather in the process known as tanning. Most tannin has molecular masses between 600-3000 KD. Tannins are general toxins that significantly reduce the growth and survivorship of many herbivores when added to their diets. Unripe fruits frequently have very high tannin levels, which may be concentrated in skin or epicarp. Interestingly, it is often desired a certain level of astringency in tannin containing foods such as apples, blackberries, tea and red wine, which are consumed as stimulatory agents in human diet (Taiz and Zeiger, 2006).

The defensive properties of tannins have been attributed to their ability to bind proteins. It has long been thought that plant tannins react with complex proteins in the guts of herbivores by forming hydrogen bond between phenolic hydroxyl groups and sites on the proteins, thus inactivating the herbivore's digestive enzymes creating complex aggregates of tannins and plant proteins that are difficult to digest. However, contradictory results have been reported by more recent experiments. A number of animals investigated possess digestive adaptations, such as detergent like compound found in the guts of some insects that appear to prevent tannins from binding proteins in their digestive systems (Martin *et al.*, 1985). Therefore, it is possible that tannins do not act simply as digestibility-reducing substances but may exert their toxicity in other ways.Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Fluck, 1973). Choudhary *et al*., (2009) in their work of antibacterial screening and

Table 26: Tannin content in different plant parts of *Aristolochia bracteolata* Lam.

Sr. No.	Plant Part	Tannin content (mg/g tannin dry tissue)
1	Leaves	1.48 ± 0.02
2	Stem	1.16 ± 0.03
3	Root	0.50 ± 0.15

Data represents average of three determinations

± indicates Standard deviation

phytochemical investigation of bark extracts of *Acacia jacquemontii* Benth. observed that stem bark extracts had alkaloids, glycosides, saponins, terpenoids alongwith tannins.

Adebajo *et al.*,(1983) stated that weed plants are rich in alkaloids, tannins and glycosides, showed antimicrobial activity against a number of micro- organisms. Also they reported that presence of tannins, glycosides and alkaloids in leaf extract of *Eugenia uniflora*.

Presence of large amount of tannins in a weed *Aristolochia bracteolata* Lam. has been recorded in present investigation. From this, it is quite clear that occurrence of more amount of tannin in this weed may play its primary role towards defense mechanism and this may be further associated with medicinal properties of this plant.

C. Aristolochic acid analysis:

Aristolochia plants have been used since ancient times in traditional herbal medicines in many parts of the world, and aristolochic acids have been reported to have antibacterial, antiviral, antifungal, and antitumor effects (Kupchan and Doskotch 1962, Zhang *et al.*, 2004). *Aristolochia bracteolata* Lam. is known as "worm killer" due to supposed antihelminthic activity and trypanocidal effect (Samia, 2006). It also possesses a potent antiallergic activity (Chitame, 2010) and has pronounced antibacterial and antifungal activities (Kavita and Nirmaladevi, 2007).

Aristolochic acids are compounds of nitrophenanthrene carboxylic acids that occur naturally in plants of the family Aristolochiaceae. Aristolochic acid content of plants or botanical preparations varies depending on the plant species, where it was grown, the time of year, and other factors. However, aristolochic acid I (also called aristolochic acid A) and its demethoxylated derivative, aristolochic acid II (also called aristolochic acid B) are the predominant forms. AA I is a crystalline solid that is slightly soluble in water. (O'Neil *et al.*, 2006). No information was located on the physical or chemical properties of AA II other than its molecular weight of 311.3 (IARC 2002).

In 2008, IARC concluded that aristolochic acids also were carcinogenic to humans (Grosse *et al.*, 2009). In contemporary medicine, *Aristolochia* plant extracts have been used in therapies for arthritis, gout, rheumatism, and festering wounds, but these uses were discontinued in Germany and other countries after the carcinogenic and

mutagenic properties of aristolochic acids were first reported in the early 1980s (Arlt *et al.* 2002). Aristolochic acids also have been used in studies of toxicity and carcinogenicity and in biochemical studies as relatively selective inhibitors of the enzyme phospholipase A2 (NTP 2008).

Due to its number of medicinal properties it is supposed to study isolation and characterization of aristolochic acid from *Aristolochia bracteolata* Lam. by using Different techniques mentioned below.

1) Quantification of aristolochic acid by different root, stem and leaf extracts of *Aristolochia bracteolata* Lam. by HPLC analysis –

The separation and quantification of aristolochic acid was achieved on HPLC system (Shimadzu, LC solution 2010) equipped with UV detector. From the chromatogram obtained (Figure 11,12,13,14,15,16) it is revealed that quantitative response of aristolochic acid in methanolic extract was more prominent as compared to ethyl acetate extract of root, stem and leaf of *A. bracteolata* Lam.

Also, in methanolic extract, maximum amount of aristolochic acid is found in root (figure 16) and stem (figure 15) followed by leaf (figure 14). Thus methanolic extract was preferable for extraction and isolation of aristolochic acid as compared to ethyl acetate extract. It justifies earlier method for isolation of aristolochic acid from *Aristolochia* species by different works as they have also used the methanol for extraction of aristolochic acid (Abdelgadir *et al.*, 2011) from *Aristolochia bracteolata,* Wu *et al*. (2000) from *Aristolochia cucurbitifolia,* Stacheno *et al.*, (2009) from *Aristolochia ringens.*

2) TLC analysis –

Methanolic extract of isolated aristolochic acid from *Aristolochia bracteolata* Lam. separated on TLC aluminum sheets silica –gel 60 F_{254} (Merk Germany) in the mobile phase- n hexane: ethyl acetate (80:20) (Plate 5). A single fraction with Rf 0.45 was obtained, which was located as a translucent yellow spot under day light. Similar studies in *Aristolochia bracteolata* Lam carried out by Abdelgadir *et al.*, (2011) they observed that aristolochic acids generally acquired black color on fluorescent silica-gel in UV-light at 365 nm while they fluoresce yellow in day light. Rf values ranged 0.43–0.46 (chloroform / methanol (6:1) ; silica-gel).

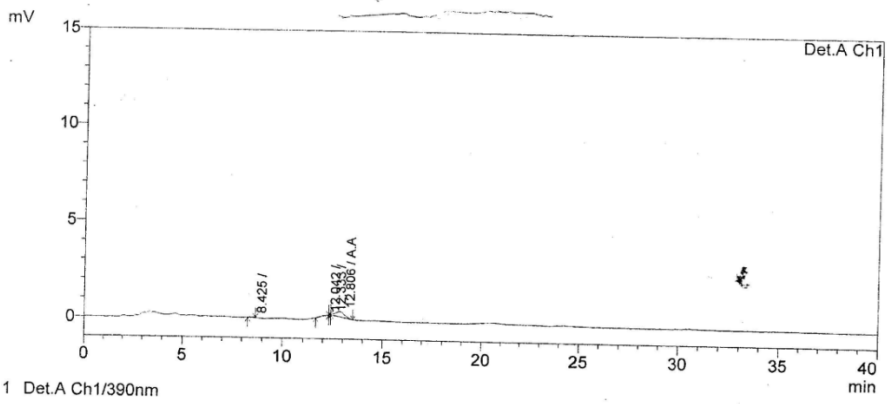
Figure 11: HPLC chromatogram of ethyl acetate leaf extract of *Aristolochia bracteolata* L

==== Shimadzu LCsolution Analysis Report ====

Tray# : 1
Vial # : 7
Injection Volume : 20 uL

Ethyl acetate Leaf

<Chromatogram>



PeakTable

Detector A Ch1 390nm

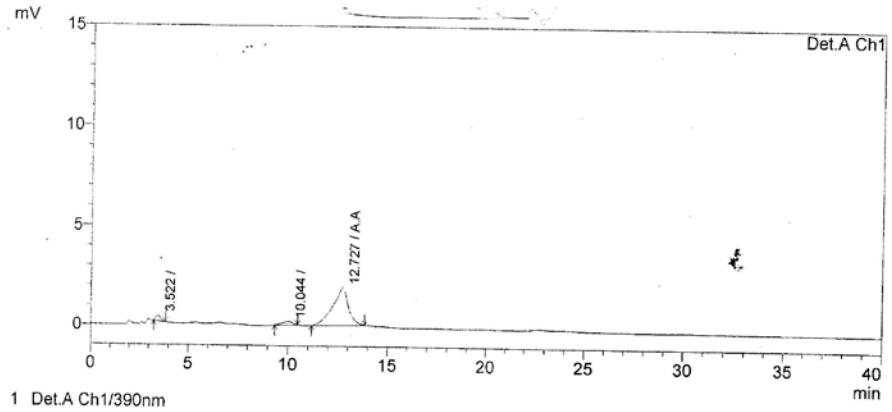
Peak#	Ret. Time	Name	Area	Area %
1	8.43		472	5.0321
2	12.04		745	7.9473
3	12.33		27	0.2828
4	12.81	A.A	8132	86.7377
Total			9375	100.0000

Figure 12 : HPLC chromatogram of ethyl acetate stem extract of *Aristolochia bracteolata* Lam.

==== Shimadzu LCsolution Analysis Report ====

Tray# : 1 Ethyl acetate
Vial # : 5 Stem
Injection Volume : 20 uL

<Chromatogram>



PeakTable

Detector A Ch1 390nm

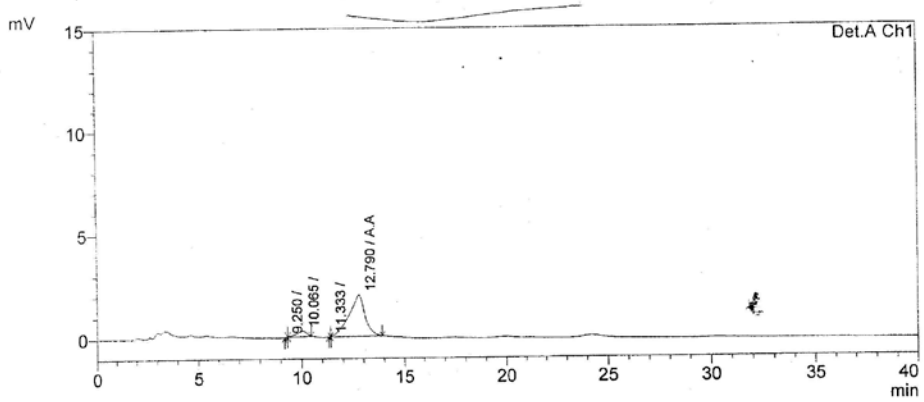
Peak#	Ret. Time	Name	Area	Area %
1	3.52		4694	4.0920
2	10.04		5754	5.0160
3	12.73	A.A	104257	90.8919
Total			114705	100.0000

Figure 13: HPLC chromatogram of ethyl acetate root extract of *Aristolochia bracteolata*

==== Shimadzu LCsolution Analysis Report ====

Tray# : 1
Vial # : 6 Ethyl acetate Root
Injection Volume : 20 uL

<Chromatogram>



1 Det.A Ch1/390nm

PeakTable

Detector A Ch1 390nm

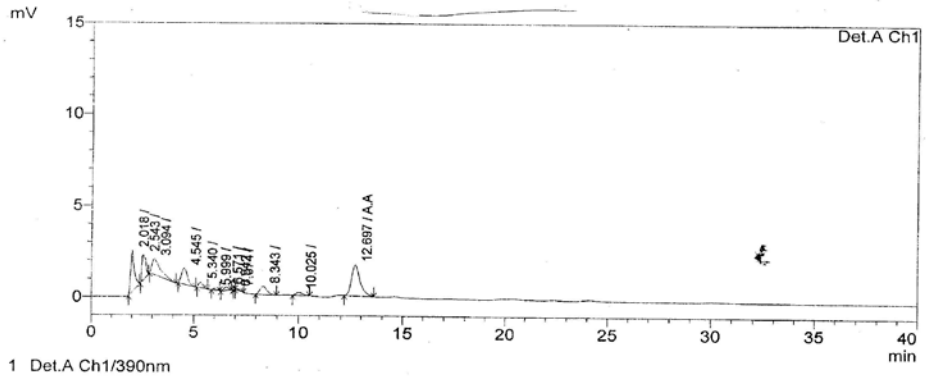
Peak#	Ret. Time	Name	Area	Area %
1	9.25		50	0.0435
2	10.06		8552	7.4814
3	11.33		23	0.0205
4	12.79	A.A	105683	92.4545
Total			114308	100.0000

Figur 14: HPLC chromatogram of methanol leaf extract of *Aristolochia bracteolata* Lam.

==== Shimadzu LCsolution Analysis Report ====

Tray# : 1
Vial # : 4 Methanol Leaf
Injection Volume : 20 uL

<Chromatogram>



1 Det.A Ch1/390nm

PeakTable

Detector A Ch1 390nm

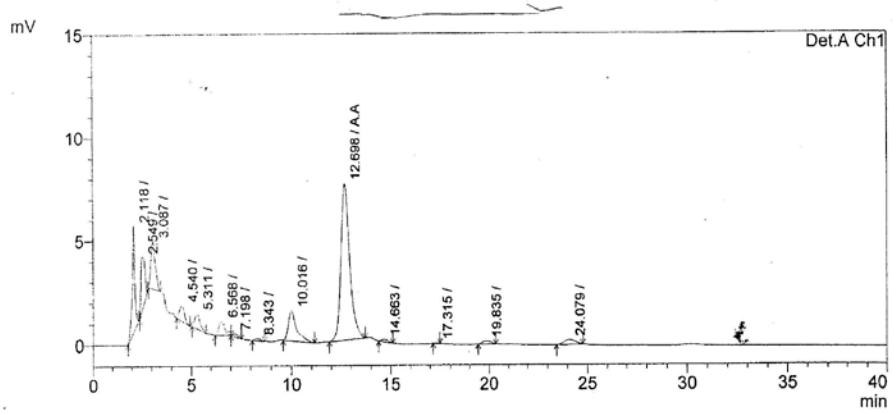
Peak#	Ret. Time	Name	Area	Area %
1	2.02		27085	17.3159
2	2.54		16651	10.6453
3	3.09		23849	15.2467
4	4.54		17334	11.0818
5	5.34		4265	2.7267
6	6.00		1077	0.6887
7	6.57		2419	1.5464
8	6.94		36	0.0228
9	7.07		1077	0.6888
10	8.34		10195	6.5180
11	10.03		3452	2.2071
12	12.70	A.A	48977	31.3117
Total			156419	100.0000

Figur 15: HPLC chromatogram of methanol stem extract of *Aristolochia bracteolata* Lam.

==== Shimadzu LCsolution Analysis Report ====

Tray# : 1 methanol.
Vial # : 3 stem
Injection Volume : 20 uL

<Chromatogram>



1 Det.A Ch1/390nm

PeakTable

Detector A Ch1 390nm

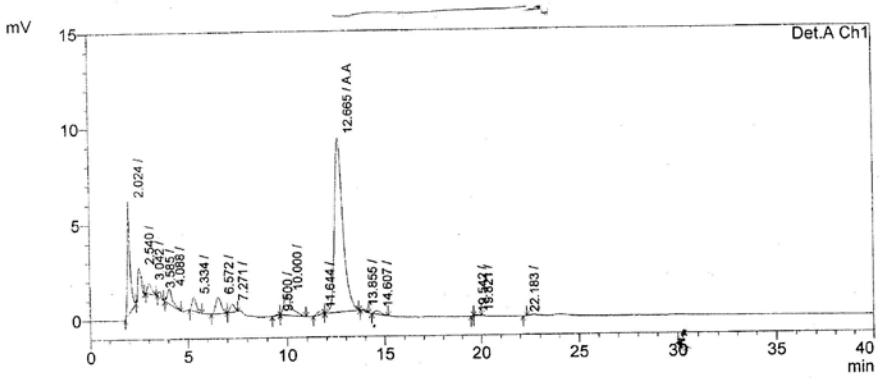
Peak#	Ret. Time	Name	Area	Area %
1	2.12		45417	10.2751
2	2.55		32648	7.3862
3	3.09		35317	7.9902
4	4.54		12800	2.8958
5	5.31		12782	2.8918
6	6.57		13131	2.9707
7	7.20		2133	0.4825
8	8.34		1712	0.3873
9	10.02		39261	8.8825
10	12.70	A.A	231795	52.4412
11	14.66		2323	0.5256
12	17.32		305	0.0690
13	19.84		3944	0.8924
14	24.08		8441	1.9098
Total			442009	100.0000

Figur 16: HPLC chromatogram of methanol root extract of *Aristolochia bracteolata* Lam.

==== Shimadzu LCsolution Analysis Report ====

Tray# : 1 Methanol.
Vial # : 2 Root
Injection Volume : 20 uL

<Chromatogram>



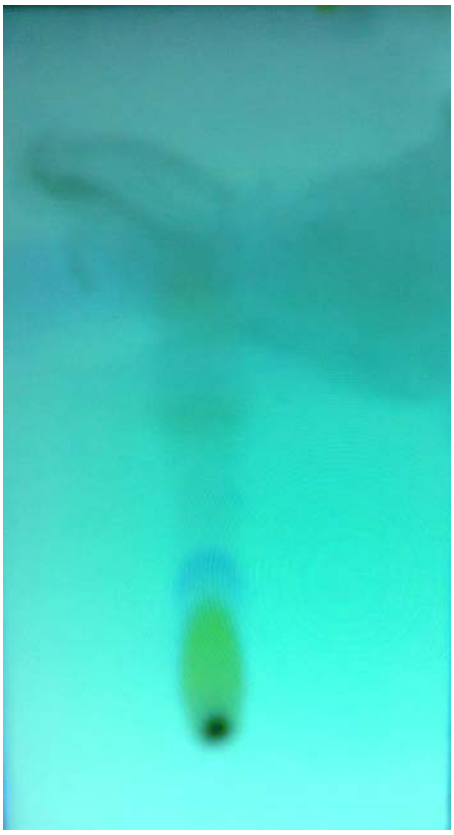
1 Det.A Ch1/390nm

PeakTable

Detector A Ch1 390nm

Peak#	Ret. Time	Name	Area	Area %
1	2.02		55698	12.6018
2	2.54		19503	4.4126
3	3.04		7725	1.7478
4	3.58		2917	0.6600
5	4.09		13237	2.9948
6	5.33		10752	2.4327
7	6.57		16285	3.6844
8	7.27		6023	1.3627
9	9.50		310	0.0702
10	10.00		28395	6.4243
11	11.64		2602	0.5887
12	12.66	A.A	272765	61.7135
13	13.85		1296	0.2932
14	14.61		3889	0.8798
15	19.54		36	0.0081
16	19.82		521	0.1178
17	22.18		32	0.0073
Total			441986	100.0000

Plate 5: TLC analysis of isolated aristolochic acid



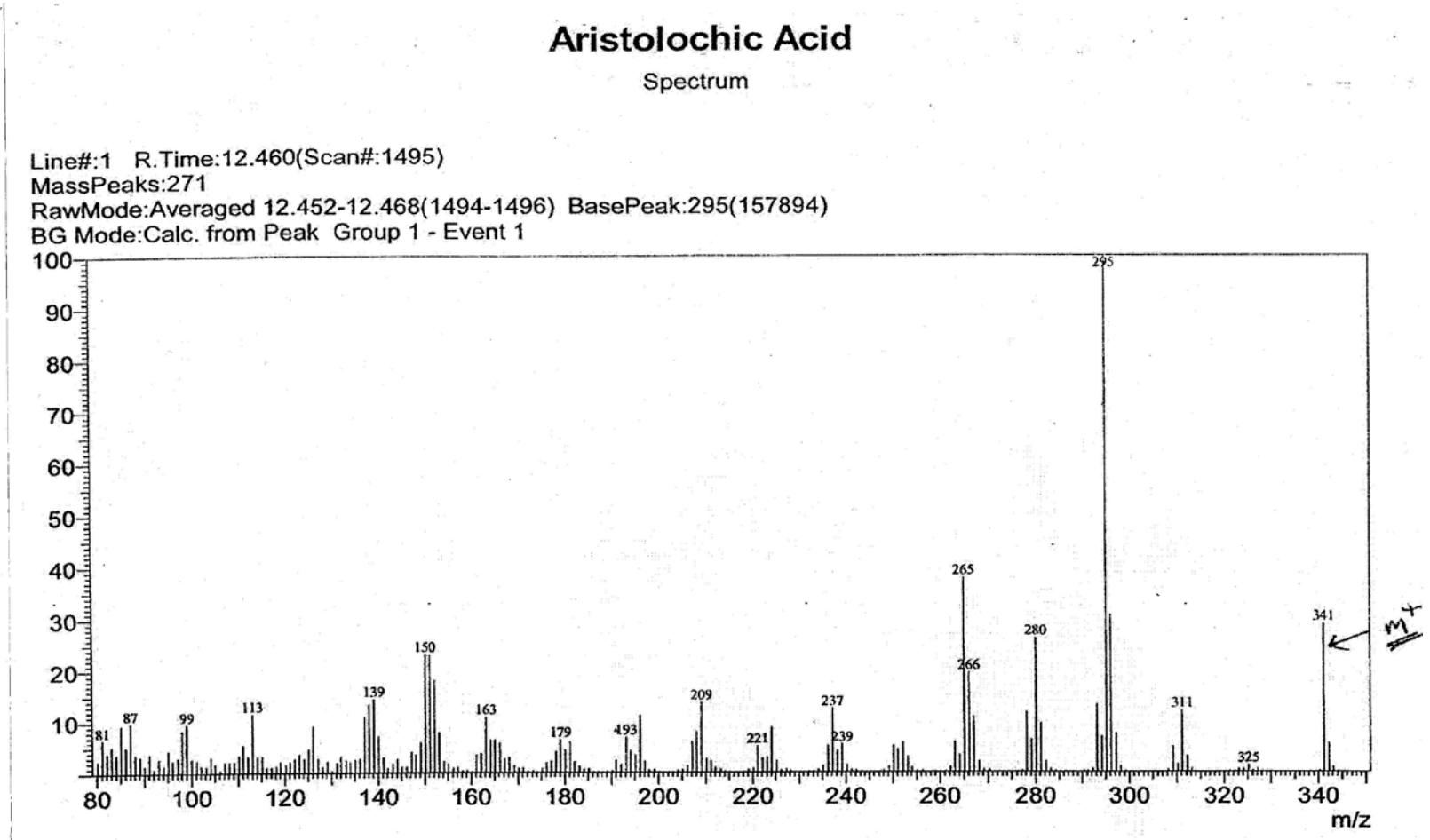
As there is single fluorescent yellow spot in day light on TLC plate. The isolated compound is aristolochic acid free from any detecTable impurities.

3) **GC-MS analysis** –

GC-MS technique is a combination of gas chromatography and mass spectrophotometery. GC (Gas Chromatography) is employed to fractionate mixture into different individual compounds with the help of thermally regulated capillary column. MS (Mass Spectroscopy) is used to identify various components on the basis of their mass spectra. Each compound has characteristics or nearly unique mass spectrum which can be compared with GC-MS database libraries developed in the last decade. It is one of the techniques able to qualitatively identify the actual nature of chemicals in the samples.

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols etc. The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (Cong *et al.*, 2007).

For GC-MS studies model Shimadzu QP 2010 was used. Extracted sample was injected through direct injection (DI) mode and Mass spectrum was recorded. Quantitative determinations were made by relative intensities of mass spectra. From the mass spectrum molecular weight of aristolochic acid was determined by selected m / z range. In full scan mass spectrum of isolated aristolochic acid, observed molecular ion base peak was at m / z 295 amu and other molecular ion peaks were at m / z 311, 280, 265, 237, 209, 179, 163 (Figure 17). Whereas from selected mass spectra observed molecular wt. is 341.20 which is comparable with theoretical molecular wt. 341.27 corresponding to CAS No. 313-67-7 (Figure 18). Fragment peak which is formed by the elimination of NO_2 group gave the base peak at m / z 295 and another fragment peak observed at 311 by the elimination of OCH_3 along with this an another fragment peak at m / z 265 by the elimination of both NO_2 and OCH_3.



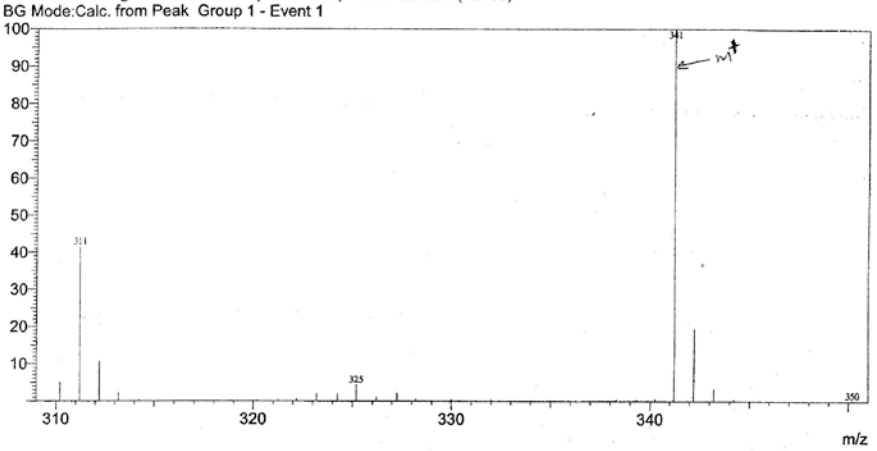
Figur e 17: Full scan M / Z GC-MS chromatogram of isolated aristolochic acid from *Aristolochia bracteolata* Lam.

Figure 18: Selected molecular mass (m / z) GC-MS chromatogram of isolated aristolochic acid from *Aristolochia bracteolata* Lam.

Aristolochic Acid

Spectrum

Line#:1 R.Time:12.460(Scan#:1495)
MassPeaks:41
RawMode:Averaged 12.452-12.468(1494-1496) BasePeak:341(45705)
BG Mode:Calc. from Peak Group 1 - Event 1



Mass Table
Line#:1 R.Time:12.460(Scan#:1495)
MassPeaks:41
RawMode:Averaged 12.452-12.468(1494-1496) BasePeak:341(45705)
BG Mode:Calc. from Peak Group 1 - Event 1

#	m/z	Abs. Int.	Rel. Int.
1	310.20	2290	5.01
2	311.20	18986	41.54
3	312.20	4913	10.75
4	313.20	1074	2.35
5	314.20	218	0.48
6	315.20	107	0.23
7	316.20	21	0.05
8	317.20	31	0.07
9	318.20	88	0.19
10	319.20	75	0.16
11	320.20	137	0.30
12	321.25	263	0.58
13	322.20	298	0.65
14	323.20	913	2.00
15	324.25	903	1.98
16	325.20	2003	4.38
17	326.20	488	1.07
18	327.25	966	2.11
19	328.20	301	0.66
20	329.00	66	0.14
21	330.00	233	0.51
22	331.00	26	0.06
23	332.00	224	0.49
24	333.00	46	0.10
25	334.00	108	0.24
26	335.00	49	0.11
27	336.00	72	0.16
28	337.00	38	0.08
29	338.30	246	0.54
30	339.30	165	0.36
31	340.25	329	0.72
32	341.20	45705	100.00
33	342.20	8903	19.48
34	343.20	1570	3.44

Our findings agreed with the results of Seto *et al.,* (2002) who have also observed molecular weight of aristolochic acid I at 341.00 and base peak at 295 by LCMS with 50% methanolic extract.

4) FT-IR analysis –

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Eberhardt *et al.,* 2007**).**The infrared region (4500-650 $^{cm-1}$) is of prime importance for the identification of functional groups as well as study of an organic compound. It has been called as 'fingerprint' of a molecule. The infrared (IR) spectrum of isolated aristolochic acid (fig. 19) showed prominent bands for the C - N stretching at 1630.04 cm^{-1}. N = O stretch signals at 1543.61 cm^{-1} symmetric stretch 1518.94 and 1595.44 cm^{-1} asymmetric stretch. Results also indicated that presence of hydroxyl group (-OH) showed strong broad signal at 2800-3700 cm^{-1}); Carbonyl group (C = O) having sharp signal at 1680.92 cm^{-1}, C-O stretching at 1262.84 cm^{-1} and C-H stretch at 748.32 cm^{-1} when compared to literature value (Kalsi,2001). Similarly, work of isolation of Aristolochic Acids from *Aristolochia bracteolata.* Saleh (2004) also showed the presence of hydroxyl group (strong broad signal at 2850 – 3579 cm^{-1}), carbonyl group (sharp signal at 1714 cm^{-1}), nitro group (signals at 1345 cm^{-1} symmetric stretch and at 1517,1594 cm^{-1} asymmetric stretch).These results justifies our obtained results and conclude that functional group absorption spectra of isolated aristolochic acid indicates that it contains the acidic(-COOH) , nitro ($NO_{2)}$ group and ether (- O - C) group.

5) **^{1}H-NMR analysis** –

In ^{1}H (NMR) spectroscopy, the characteristic absorption of energy by certain spinning nuclei in a strong magnetic field, when irradiated by a second and weaker field perpendicular to it, permits the identification of atomic configurations in molecules with respect to the number of different kinds of hydrogen atoms in the molecule.

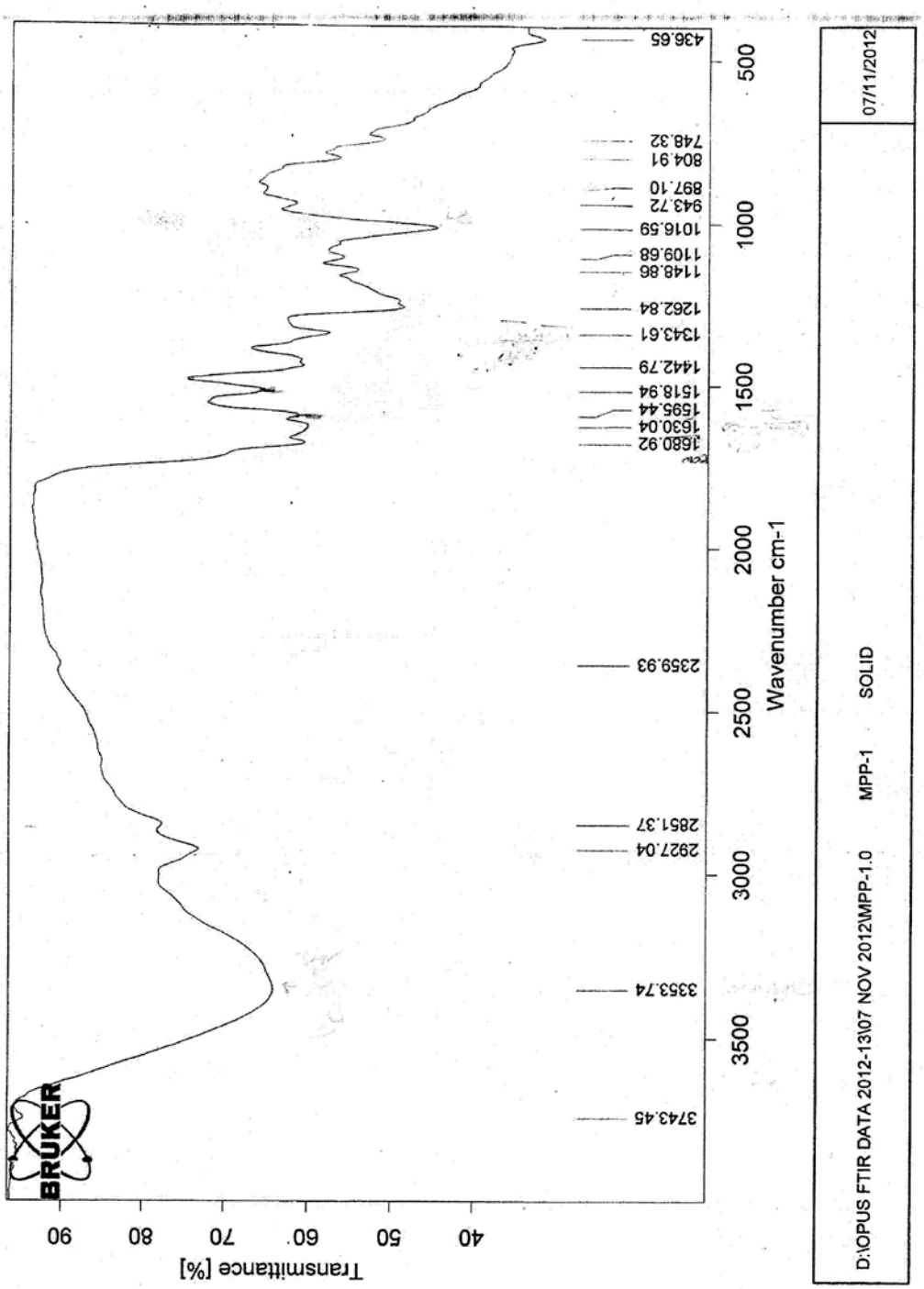


Figure 19: FT-IR spectrum of isolated aristolochic acid from *Aristolochia bracteolata* Lam.

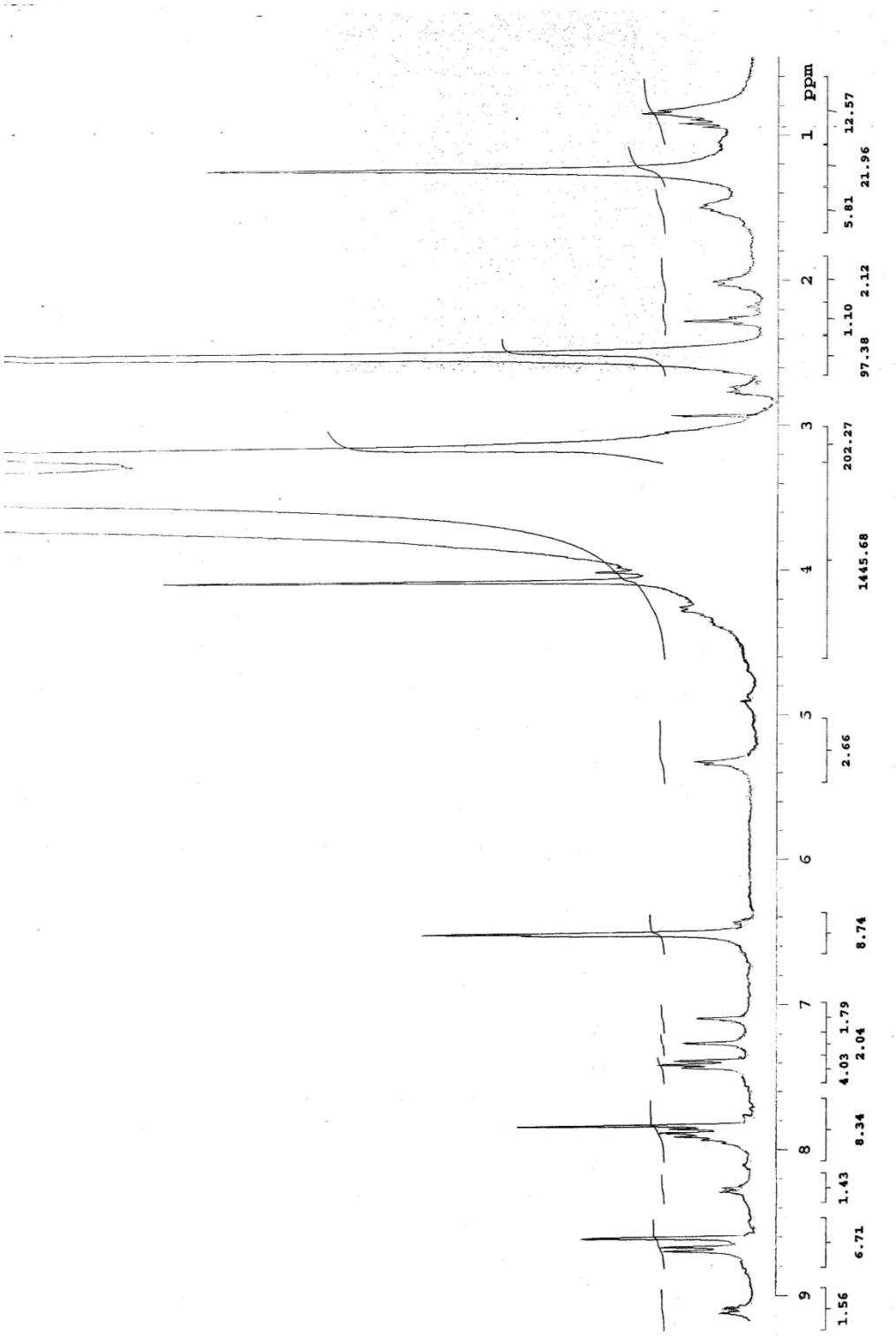


Figure 20 : NMR spectrum of isolated aristolochic acid from *Aristolochia bracteolata* Lam.

Table 27 : NMR spectral data of isolated Aristolochic acid from *Aristolochia bracteolata* Lam.

Substituent group	Chemical shift (δ in ppm)	Pattern of peak / signals
Proton –Aromatic H_5	7.928	Doublet
Proton –Aromatic H_7	7.258	
Proton –Aromatic H_2	6.433	Singlet
Adjacent NO_2 group H_9	8.664	
-CH_3O group	4.248	Singlet
-OCH_2O- group	5.334	

The ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectrum of isolated aristolochic acid is recorded in Table 27 and fig. 20. From the Table it is observed that it showed signals at δ 7.258 and δ 7.928 attributed to H_7 and H_5 respectively. Two singlets at δ 6.433 and δ 8.664 were assigned to C2 and C9 protons. Presence of methoxyl group at δ 4.248 and methylenedioxy group at δ 5.334.

6) **HPLC analysis** –

High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products (Cannell, 1998). Purification of the compound of interest using HPLC is the process of separating or extracting the target compound from other (possibly structurally related) compounds or contaminants. Each compound should have a characteristic peak under certain chromatographic conditions. Depending on what needs to be separated and how closely related the samples are, the chromatographer may choose the conditions, such as the proper mobile phase, flow rate, suitable detectors and columns to get an optimum separation (Sasidharan *et al.*, 2011).

A reverse phase HPLC analysis was conducted on Shimadzu LC 2010 chromatic system HPLC as shown in chromatogram (figure 21) using Methanol: Water: Acetic Acid 67:32:1 as mobile phase. Aristolochic acid detected, major peak of component as a R.T. was about 5.7 min. Purity of isolated Aristolochic acid performed on High Pressure liquid Chromatography. Isolated aristolochic acid purity observed as about 75.00 %.

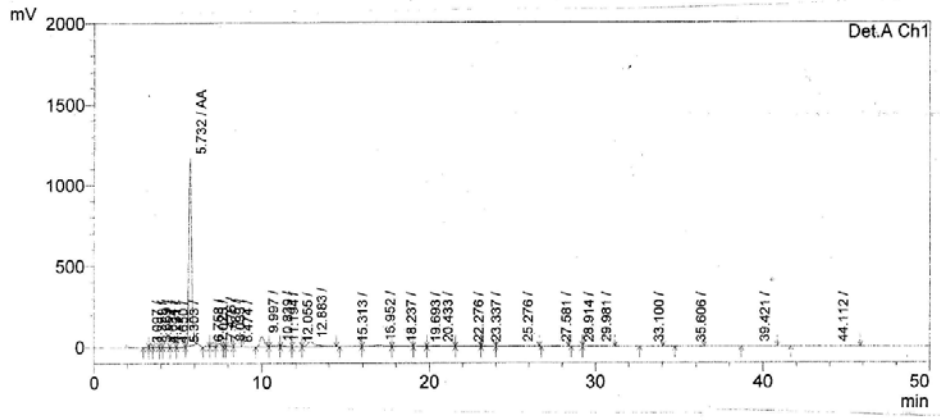
7) **X-ray crystallography analysis** –

Single crystals of the compound were grown by slow evaporation of the solution in MeOH. Crystals of aristolochic acid were studied by analyzing the X-ray diffraction patterns obtained using Philips X-ray diffractometer Model PW 3710 for λ = 1.05406 and 2.2897 A^0 for Cu- K_α and Cr-K_α radiation, M = 341.27, $C_{17}H_{11}NO_7$. Results of X- ray analysis of isolated Aristolochic acid are depicted in fig.22) .Crystals belong to monoclinic, space group P21/C, *a* (A^0)= 17.9200, *b*

Figur 21: HPLC chromatogram of isolated aristolochic acid from *Aristolochia bracteolat*

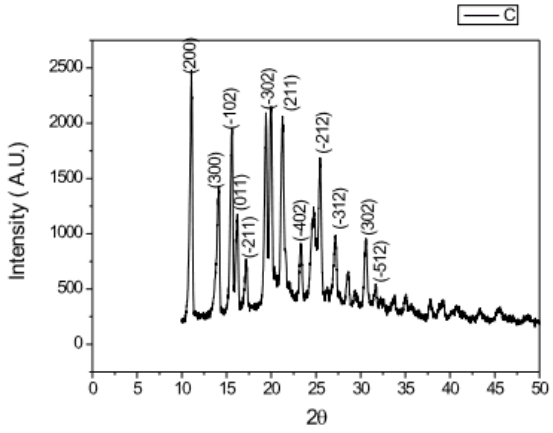
==== Shimadzu LCsolution Analysis Report ====

<Chromatogram>



<Results>

Peak #	Name	Ret. Time	Area	Area %	Relative Retention Time
1		3.10	5776	0.03	0.54
2		3.40	5922	0.03	0.59
3		3.67	62417	0.36	0.64
4		4.03	13425	0.08	0.70
5		4.23	39395	0.23	0.74
6		4.65	10699	0.06	0.81
7		5.30	19479	0.11	0.93
8	AA	5.73	13125198	75.02	1.00
9		6.76	80112	0.46	1.18
10		7.02	66726	0.38	1.23
11		7.48	262692	1.50	1.30
12		7.79	31136	0.18	1.36
13		8.03	121521	0.69	1.40
14		8.47	29530	0.17	1.48
15		10.00	1132104	6.47	1.74
16		10.84	288611	1.65	1.89
17		11.19	127235	0.73	1.95
18		12.05	24603	0.14	2.10
19		12.88	1076080	6.15	2.25
20		15.31	36028	0.21	2.67
21		16.95	353860	2.02	2.96
22		18.24	75595	0.43	3.18
23		19.69	36613	0.21	3.44
24		20.43	140762	0.80	3.56
25		22.28	65305	0.37	3.89
26		23.34	15666	0.09	4.07
27		25.28	52263	0.30	4.41
28		27.58	22867	0.13	4.81
29		28.91	3274	0.02	5.04
30		29.98	57567	0.33	5.23
31		33.10	4474	0.03	5.77
32		35.61	17175	0.10	6.21
33		39.42	23730	0.14	6.88
34		44.11	67029	0.38	7.70
Total			17494871	100.00	



Crystallographic parameters

Crystal system:	Monoclinic
Space group:	P21/c
Space group number:	14
a (Å):	17.9200
b (Å):	5.2540
c (Å):	9.5700
Alpha (°):	90.0000
Beta (°):	106.2700
Gamma (°):	90.0000

Figure 22 :X-ray analysis of isolated aristolochic acid from *Aristolochia bracteolata* Lam.

(A^0) = 5.2540, *c* (A^0)= 9.5700 (A^0), α=90.0000(A^0), β=106.2700 (A^0), γ = 90.000 (A^0), Data collection and refinement parameters are listed in Table of peak search .

All characterization parameters showed that isolated compound is aristolochic acid A. acid I (8-methoxy-6-nitrophenanthro [3, 4-*d*][1, 3] dioxole-5-carbosylic acid) with molecular formula $C_{17}H_{11}NO_7$ with molecular weight 341.27.

D. Effect of water stress

a) Leaf Water Status :

The influence of water stress on different parameters of leaf water status like moisture content, succulence index, mesophyll succulence index and surface expansion in *A. braceteolata* Lam. leaves is recorded in Table 28. It is clear from the results that all above mentioned parameters shows consistent decline in their values due to increasing water deficit.

1. **Moisture Percentage:**

It is evident that water stress has affected moisture content in *A. braceteolata* Lam. leaves. As it shows decrease from 89.11% to 79.41% with increasing drought conditions. It is interesting to note that even at moderate and severe water deficiency the moisture content has not decreased significantly and it seems that high moisture content has been maintained even in water deficit conditions.

Water is the most abundant material in a growing plant. The weight of water in a plant is usually four to five times the total weight of dry matter. It is a solvent for mineral nutrients and complex substances manufactured within the plant. It also acts as transportation agent and the means whereby the equilibrium of salts and other dissolved products is maintained between various plant parts. Alongwith this it is a raw material for the process of photosynthesis-the basic process underlying all life.

The other important functions of water in plant include: maintaining cell turgidity for structure and growth; transporting nutrients and organic compounds throughout the plant, comprising much of the living protoplasm in the cells, serving as a raw material for various chemical processes, including photosynthesis; and, through

Table 28 - Parameters of Leaf Water Status in *Aristolochia bracteolata* Lam. under Water Stress Conditions.

Sr. No	Parameter	a	b	c	d
1	Moisture content (%)	89.11 ± 0.04	87.94 ±0.6**	80.31±0.05*	79.41±0.4
2	Succulence Index $g.g^{-1}$	9.18 ±0.02	8.29±0.05*	5.66 ±0.06*	4.86±0.06
3	Mesophyll Succulence index(Sm)g mg^{-1}chl	0.48 ±0.04	0.35±0.05*	0.33±0.00*	0.31±0.00
4	Surface Expansion m^2kg^{-1}	8.22 ±0.00	7.97±0.02*	5.44±0.00*	3.38±0.02

(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1 days no water

(c) 48 hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with 3days no water

* - Values are significant at 5% level where $P < 0.05$.

transpiration, buffering the plant against wide temperature fluctuations etc.

Modifications of plants in relation to water environment are one of the important factors determining areas of adaptation of those plants. These modifications are also among the chief factors which determine management requirements of various plants. One of the important, but less frequently mentioned, functions of water is that of temperature control as the water vapour given out by leaves through transpiration produces a cooling effect. This cooling effect helps to maintain a favorable temperature around the leaves of the plant.

Hsiao and his coworkers (1998) proposed that the role of cell turgor or tissue water status in determining leaf growth rates. The results showed that growth rate changed with changes in plant water status when the water status was low, but was unaffected when water status was very high in maize leaves. These findings are consistent with those of by Munns *et al* .(2000) in their study on water relations and leaf expansion. They observed that, leaf water status could limit leaf expansion rates during periods of high transpiration despite the control exercised by hormonal effects of which on a 24 h basis in barley and maize leaves.

Reduction in moisture level in plant tissue is essentially first detectable change caused by water deficit. However, this reduction in moisture uptake varies from plant to plant. Leaf is the organ most responsive to environmental conditions and also it's structure reflects effect of water stress more clearly than that of stem and roots (Nevo *et al*., 2000).

Salunkhe (1990) had reported decrease in moisture content in leaf, stem and root of *Dodonea viscosa* L. plant under water deficit. Similarly, Jadhav (2000) noticed that in prosomillet leaves even after 16 day water stress, about 23% moisture percentage was recorded as the leaves were almost dry and rolled. There was a significant decline in leaf RWC alongwith decrease in moisture level from 75% to 25% field capacity of nine cultivars of wheat (Razzaq *et al*., 2013).

It is well evident from the present findings that moisture content is moderately high at normal conditions in this weed. This obviously reflects the succulent mature of this plant. Further, with increasing water stress also, moisture content shows a very meagre decline in status, thereby showing its ability to maintain water status even under water deficiency. This may be one of the reasons for its success to survive under arid, dry and hot environmental conditions.

2. Succulence Index :

The effect of water stress in succulence Index (SI) of *A. braceteolata* Lam. is recorded in Table 28. It is evident from the results that succulence index decreased drastically from 9.18 g. g^{-1} to 4.86 g. g^{-1} with increasing water stress.

Drought caused considerable morphological changes in leaves of sugerbeet such as increased specific leaf weight and succulence index (Vahidi *et al*., 2013) which were related to leaf thickness (Hampe and Marschner, 1982). Such leaf response to water stress was also reported by Ober and his coworkers in sugerbeet (2005). Kluge and Ting, (1978) proposed that the succulence index for whole thick leaves and their mesophyll was typical of CAM plants.

The close relationship between succulence and leaf thickness has also been reported by Bussoti *et al.*, (2002) as thicker leaves of *Quercus ilex* showed a greater volume of water per surface area. Out of five olive cultivars grown under drought conditions, Blanqueta, Negrinh and Manzonilla cultivars showed high succulence index, the parameter to be used as kind of protection (Bacelar *et al.*, 2004). Leaf and stem succulence is a common feature among species of *Peperomia* and suggests a role for stored water in the water economy of these plants. Water movement from the thick to the thin leaves of succulent plants has been shown to be important in maintaining metabolism (Tuffers *et al.*, 1995).

Razzaq *et al.*,(2013) in his study of drought resistance index of nine wheat cultivars under different moisture conditions observed that wheat cultivar chakwal- 50 was found to maintain its high RWC and succulence than other cultivars It also affecting productivity even at low levels of soil moisture. Thus concluded that high succulence can protect the plant from sudden wilting and severe leaf shrinkage.

Decrease in soil water potential causes decline in succulence and this may be an adaptation to resist drought. Highest succulence indicates high relative water content and succulence under drought may be due extensive growth of root system in this weed showing thereby tendency to maintain water supply to shoot (Qi *et al.,* 2009).

Thus, also in water stress, maintenance of succulence in leaves of *Aristolochia bracteolata* Lam. may be to conserve water when it is unavailable. This may because of occurrence of crassulacean acid metabolism in leaves of *Aristolochia bracteolata* Lam. which was reported by Deshmukh and Murumkar (1996).

3. Mesophyll Succulence Index :

Effect to water stress with respect to mesophyll succulence index in leaves of *A. braceteolata* Lam is shown in Table 28. It is clear from the Table that there is very small decline in its values of Mesophyll Succulence Index (MSI) from 0.476 g mg^{-1}chl to 0.308 g mg^{-1}chl with increasing water stress and almost the mesophyll succulence index has been maintained by this weed even under water deficit conditions. The normal condition has shown high MSI value indicating succulent nature of this weed.

Succulence is due to the presence of non-photosynthetic water accumulating tissues and in CAM plants survival may be increased thanks to increased water use efficiency and succulence, which may delay dehydration (Herrera, 2009). According to Landrum (2002) leaf succulence is common in plants subject to frequent drought and it evolved in diverse phylogenetic lineages worldwide. Kluge and Ting (1978) who proposed “mesophyll succulence” index (Sm), considering that previous indices underestimated the capacity of photosynthesizing cells to store water. “Sm” is the ratio of water content/chlorophyll content of the chlorenchymatous tissue. Ripely *et al.,* (2013) postulated that increasing leaf succulence might be associated with decreasing mesophyll conductance and increasing dependence on Crassulacean acid metabolism (CAM).

Water movement from the thick to the thin leaves of succulent plants has been shown to be important in maintaining metabolism (Schafer and Luttge, 1987). According to Kluge and Ting, (1978) one variable that has proved useful in assessing operation of CAM is the mesophyll succulence index, Sm, i.e. the ratio of leaf water : mg leaf

chlorophyll; using this parameter a plant can be considered CAM nature. In the inducible species *C. trifoliata* (Olivares *et al.*, 1984), *T. triangulare* (Herrera *et al.*, 1991) and *T. paniculatum* (Guerere *et al.*, 1996), Sm was always higher than one. The determination of Sm in the photosynthetic tissues of facultative CAM species helps to establish the relationship between succulence and facultative CAM.

Herrera and his coworkers (2000) in his study on *Pepromia carnevalli* observed that upto 40 days of drought, the succulence index changed from 6.0 to 3.0 g mg^{-1}chl for whole thick leaves and from 1.4 to 2.9 g mg^{-1}chl for the mesophyll alone. Once positive gas exchange had ceased the capacity for photosynthesis was maintained for longer periods of drought and this was directly correlated to the degree of leaf succulence.

Thus, only negligible decrease in mesophyll succulence index in *Aristolochia bracteolata* Lam. at water stress can be correlated to facultative CAM nature as well as sustainance of high succulence in this weed.

4. Surface expansion:

Effect of water stress on surface expansion in leaf tissue of *Aristolochia bracteolata* Lam. is depicted in Table 28. It is evident him the figure that water stress has caused a significant decline in surface expansion values. At severe water stress it decreased to 3.39 m^2kg^{-1} when compared with control (8.22 m^2kg^{-1}).

Leaf expansion is the central process by which plants colonize space, allowing energy capture and carbon acquisition. Water and carbon emerge as main limiting factors of leaf expansion, but the literature remains controversial about their respective contributions and is a major component of plant performance. It enables light capture, which powers photosynthesis and thus biomass production. It is also one of the first plant functions affected by environmental stresses such as water deficit (Hsiao, 1973), making it a key target for identifying tolerant genotypes and species (Tardieu and Tuberosa, 2010). Thus understanding the processes dominating the control of leaf expansion is a crucial step.

Leaf expansion is predominantly driven by cell turgor, itself largely induced by soil water potential and transpiration. Notably, increasing soil water deficit or evaporative demand leads to growth inhibitions that correlate in space and time with turgor

depressions in the elongating zone of maize (*Zea mays*) leaves was reported by Ehlert *et al.*, in 2009.

Chaves *et al*., (2002) suggested that younger leaves tend to be more resistant to drought than older leaves and this increased tolerance may be particularly relevant in plants where a sever reduction in size of leaf canopy occurs as a result of shedding of older leaves. Decreased availability of the substrate to the mitochondria under conditions of low photosynthesis as well as inhibition of leaf growth may explain reduced respiration mostly in its growth component (Gimenez *et al.*,1992).

Water stress reduces number of grain at preanthesis stage and largeness (especially for genes) of numeral morphological and domestication related traits such as plant height, flowering time, leaf morphology in wheat (Farooq *et al.,* 2011). Severe water deficit resulted in a strongly reduced expansion during day phases and a practically unaltered expansion during nights in *Arabidopsis thaliana* starch mutants. This result is relevant to recent review of (Muller *et al.,* 2011) showing that drought improves the carbon status of plants due to a higher sensitivity to drought for leaf growth than for carbon assimilation, leading to carbon accumulation.

Recently Lisar, (2012) observed decreased leaf growth, total leaf area and leaf area plasticity under drought condition in many plant species such as peanut and *Oryza sativa*.

In present findings surface expansion values dropped from 8.205 m^2kg^{-1}at the beginning of experiment to 3.382 m^2kg^{-1} in *Aristolochia bracteolata* Lam. leaves and these values are in accordance with those reported for CAM plants (Walter, 1926). This feature points clearly towards CAM nature of this weed under drought.

b) Mineral nutrition under water stress conditions:

Distribution and uptake of various inorganic constituents such as Nitrogen, phosphorous, potassium, calcium, magnesium, sulphur, sodium, iron, manganese, zinc, copper in different parts of *Aristolochia bracteolata* Lam. grown under normal and drought stressed conditions have been recorded in Table 29. It is clear from the Table that

water stress markedly altered the composition of the plant with respect to various inorganic nutrients.

1. Nitrogen:

Nitrogen content in leaf, stem and roots of *Aristolochia bracteolata* Lam. is presented in Table 29. The same Table also depicts the effect of water stress on nitrogen content in these parts. In *Aristolochia bracteolata* Lam. highest N content is recorded in leaf (241.31 mg/g) followed by stem (109.95 mg / g) and root (79.96 mg / g). Thus, in this plant leaf showed highest N content indicating it's accumulation and utilization. The water stress showed overall accumulation of N with increasing water stress. The stress showed significant rise in N content in leaf followed by stem while roots show marginal increment. As per the Table, it is interesting to note that early water deficit stress for two days has showed decrease in N content and this impact is more clear in leaf and stem samples, with increasing water deficit (172 hrs) again there is overall trend of accumulation of N content i.e. leaf from 241.31 to 272.72 mg/g and in stem 109.95 to 144.93 mg / gm. It is well evident from the Table that even with water stress the general trend of N accumulation has remained the same i.e. leaf N content has shown highest values while the lowest one's are recorded in roots.

Total nitrogen content provides an idea of overall nitrogen status in plant tissue. It is a fundamental constituent of amino acids, proteins and nucleic acids. It is invariably found in essential compounds such as IAA, cytokinins and in many of vitamins. It is a major component of chlorophyll (Goodwin, 1976). As nitrogen is an essential constituent of amino acids and hence the proteins. It cannot be absorbed by the plants directly from the atmosphere though it contains 79% of nitrogen. Plants can absorb nitrogen only in the form of salts of nitrogen. It is the most important mineral nutrient for plants. Since plants require the greatest quantity of N of all the mineral elements, deficiency of N is a limiting factor for plant growth. Complex interactions via biochemical networks of metabolite pathways exist in many aspects of N metabolism. N metabolism involves N uptake and

Table 29 - Inorganic Elements * in different parts of *Aristolochia bracteolata* Lam. under water stress conditions.

Sr. No	Inorganic Element	Leaf				Stem				Root			
		a	b	c	d	a	b	c	d	a	b	c	d
1	Nitrogen	241.31	234.88	299.14	272.72	109.95	92.81	120.66	144.93	79.96	86.39	78.53	10.35
2	Phosphorus	51.66	58.12	90.4	90.4	38.74	48.43	64.57	80.72	32.29	41.97	41.97	48.43
3	Potassium	49.62	47.32	84.4	74.17	31.97	35.04	68.8	98.22	17.14	23.02	17.91	19.95
4	Calcium	71.61	70.61	46.66	31.19	31.1	31.94	33.69	26.2	17.72	15.22	17.22	17.97
5	Magnesium	20.57	16.46	21.4	21.39	13.58	15.64	13.58	18.93	15.23	17.28	8.23	14.81
6	Sulphur	71.73	74.85	64.55	87.32	40.54	26.82	32.74	71.73	34.3	28.38	37.42	56.13
7	Sodium	86.96	86.9	56.53	50	56.53	65.66	51.74	59.57	25.65	34.79	16.52	21.74
8	Iron	12.53	14.36	9.31	14.81	12.89	19.66	13.47	14.5	13.57	16.42	17.33	19.63
9	Manganese	0.95	1.22	0.58	0.96	0.73	0.82	0.29	0.42	0.56	0.62	0.8	1.46
10	Zinc	4.47	1.59	1.38	1.54	1.76	3.85	1.12	2.43	0.8	1.7	1.59	3.21
11	Copper	0.61	0.22	0.16	0.14	0.36	0.17	0.14	0.17	0.27	0.27	0.27	0.47

*- mg /gm dry matter

(a) Control – Plants in pots watered everyday. (b) 24hrs –Plants in pots with 1 days no water

(c) 48hrs - Plants in pots with 2 days no water (d) 72hrs -Plants in pots with 3 days no water

regulation, N reduction and signaling, amino acid metabolism and transport, interactions between N and C metabolism, and the translocation and remobilization of N (Krouk *et al.,* 2010).

There is extensive literature available regarding the effect of water stress on nitrogen metabolism. Nitrogen absorption and utilization by plants under water stress is very critical for plant growth and productivity. Nitrogen affects carbon partition and improves accumulation of soluble sugars especially starch which in turn improves leaf growth (Rufty *et al.,* 1988). Under water stress conditions, the uptake of N decreased in soybean plants (Tanguilig, 1987). This decline in N uptake in shoot had been attributed to the decreased transpiration rate to transport N from root to shoot. Nitrogen deficiency, sensitized cotton plants to water stress, caused the effect of stress to occur at a higher water potential. The common opinion extended by Barnett and Naylor (1966) holds that the high N level of crop plants subjected to water stress is primarily due to the fast accumulation of free amino acids that are not converted into proteins.

Wang and Gas (2004) observed that total nitrogen content of root, stem and leaf of bald cypress increased with increasing of soil water content. Utrillus *et al.,* (1995) studied seasonal changes in production and nutrient content of *Cynadon dactylon* subjected to water stress and concluded that nitrogen showed low concentration during summer and increased in autumn with stress.

On the contrary, Dina and Klikoff in 1973 recorded that in Big Sagebrush leaf nitrogen content significantly decreased in water stressed plants, while stem nitrogen content significantly increased. Nikam (2007) also observed that in *Chlorophytum borivilinum,* nitrogen nutrition is quite sensitive to water stress. Slight water stress (PEG $\leq$ 5%, Water potential $\geq$ - 0.05 MPa) has marginal effects on contents of amino acid nitrogen, soluble protein and activities of nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) and moreover the absorption and accumulation of nitrate nitrogen was stimulated in rice seedlings (Yuan-Yuan *et al.,* 2009). Osuagwu and Edeoga, (2012) postulated that increase in nitrogen content due to water stress in the leaves of *Gongronema latifolium* might have been due to mobilization nitrogen to the leaves for the synthesis

of needed amino acids and proteins to enable the plants to withstand the effect of water stress.

In present study, high content of N in leaf signifies its high metabolic role in synthesis. However, short term water stress has stimulated proteolytic activity. Thus, showing slight decline in N content. At severe water stress, there is accumulation of nitrogen. This may be due to accumulation of N in free amino acids like proline. This supports with the data from proline accumulation in *Aristolochia bracteolata* Lam. in water stress.

2. **Phosphorus**:

Phosphorus content in leaf, stem and roots of *Aristolochia bracteolata* Lam. are presented in Table 29. The same Table also depicts the effect of water stress on phosphorus content in these parts. In *Aristolochia bracteolata* Lam. highest P content is recorded in leaf (51.66 mg/g) followed by stem and root (32.29 mg/g). Thus, in this plant leaf showed highest phosphorus content indicating it's accumulation. The water stress showed overall accumulation of P with increasing water deficit. The stress showed significant rise in phosphorus content in leaf followed by stem, while roots showed marginal increment. It is interesting to note in leaf and stem with increasing water deficit (72 hrs) again there is overall accumulation trend of phosphorus content i.e. leaf from 51.66 to 90.40 mg/g and in stem 38.74 to 80.72 mg/g. From figures it is obvious that the concentrations of phosphorus in leaf, stem and root are increased with water stress. Among the various plant parts, however, the increase in phosphorus is quite significant in leaf followed by stem and root. This indicates that probably the transport of P metabolites from the leaf to stem and finally to the roots.

Phosphorus is an essential element for plant growth, as it is involved in most of the metabolic processes. Phosphorus is a constituent of nucleic acids, phospholipids, phosphoproteins, dinucleotides, and adenosine triphosphate. Thus, P is required for storage, transfer of energy, photosynthesis, regulation of some enzymes and transport of translocates. Phosphorus is phloem mobile, and the proper symptoms of P deficiency appear first in old leaves as P is redirected to young leaves. In plants, unlike nitrate and sulphate, phosphorus is not reduced but remains in its highest oxidised form. It is present in plant cells either as inorganic phosphorus, phosphate ester or energy rich phosphate bonds.

Water-stress conditions of the growth media has far-reaching effects on the uptake and concentration of phosphorus by crop plants. Gerakis *et al*., (1975) pointed out that of the many studies conducted before about the mid 1950s, 12 of the 21 papers reported water stress decreased P concentrations in plants, and 9 papers reported that water stress did not affect the P status of plants. Since then studies have reported both decreases (Hoffman and Jungk, 1995) and increase in P levels and other mineral element concentrations with increased water stress. The positive effects of phosphorus on plant growth under drought have been attributed to an increase in stomatal conductance (Bruck *et al.,* 2000), photosynthesis (Ackerson, 1985), higher cell membrane stability, water relations (Sawwan *et al.,* 2000).

According to Jadhav (1984) in prosomillet root stem, flag and mature leaves phosphorus showed accumulation. Similar results also obtained by Salunkhe (1990) that phosphorus uptake in *Dodonea viscosa* stem is quite significant followed by root. According to Premchandra *et al*.,(1995) the concentration of Phosphorus was increased with increasing level of water stress in *Sorghum* grains. Increase in phosphorus level is observed in the tubers of water stressed plants of *Chlorphytum borivilinum* by Nikam (2007).

Whereas, on the other hand, earlier Wilson *et al.* (1968) observed that reduction of phosphorus content due to drought was a common feature in many annual forage legumes and reduction was of the order of 4 to 68%. Samuels (1972) had reported that water stress greatly hampered phosphorus uptake process. Reduction in phosphorus uptake due to drought leads to further metabolic disturbances as phosphorus is essential for many processes. Many workers observed decrease in phosphorus uptake due to drought to quote few of them Kirnak *et al*.(2001) in egg plant, Utrillas *et al.,* (1995) in *Cynadon*. In drought-treated beech seedlings the level of phosphate was reduced and this was attributed as its function in charge balance (Peuke and Rennenberg, 2011).

While Osuagwu and Edeoga (2012) observed that water stress has no significant effect on phosphorus content in leaves of *Gongronema latifolium*.

From the above discussion and with results obtained for phosphorus content in *Aristolochia bracteolata* Lam., it is obvious that the phosphorus content in leaf, stem and root has shown increase with prolonged water stress. The enhanced values of P in

shoot that is leaf and stem reflect its lower mobility, accumulation of P compounds and enhanced proteolysis. This might be adaptive feature to overcome drought.

3. Potassium :

Potassium content in leaf, stem and roots of *Aristolochia bracteolata* Lam. are presented in Table 29. The same Table also depicts the effect of water stress on potassium content in these parts. In *Aristolochia bracteolata* Lam. highest K content is recorded in leaf (49.62 mg/g) followed by stem (31.97mg/g) and root (17.14 mg/g). The water stress showed overall accumulation of potassium with increasing deficit. It showed significant rise in potassium content in stem followed by leaf while roots show very small increment. In leaf and stem with increasing water deficit (72 hrs) there is overall accumulation trend of potassium content i.e. leaf from 49.62 to 74.17 mg/g and in stem 31.97 to 98.22 mg/g. It is obvious from the figures that the concentrations of potassium in leaf, stem and root is increased with water stress. Among the various plant parts, however, the increase in potassium is quite significant in stem followed by leaf and root. This indicates that probably the hampered transport of potassium from the stem to leaf and roots.

Potassium is the most abundant ion in the plant cell and is required for wide array of functions, ranging from maintenance of electrical potential gradients cell across membranes, to the generation of turgor, to the activation of numerous enzymes. The majority of these functions depend more or less directly upon the activities and regulation of membrane bound K^+ transport proteins, operating over a wide range of K^+ concentrations. Epstein (1972) has suggested that the potassium level in plant tissue for plant growth is 1% dry weight. Potassium maintains osmotic pressure turgor of the cells (Lindhauer, 1995) and regulates the stomatal functioning under water stress (Kant and Kafkafi, 2002). It is the most abundant cation in the cytosol, xylem and phloem sap (Peuke, 2010), but it can be functionally replaced in the vacuole by other cations (Marschner, 1995).

Present findings are in agreement with those Egila *et al.*,(2001) who has summarized that Potassium enhances photosynthetic rate, plant growth and yield under stress conditions Similar observations have been made latter by Jadhav (1984) in root and to some extent in flag leaf of prosomillet. Salunkhe (1990) observed that potassium content in the roots of *Dodonea viscosa* L. is doubled due to water stress

but no change in stem potassium content while in leaves there was decline in potassium content. Accumulation of K in shoots of drought stressed plants of all three cultivars of tall fescue was observed by Huang (2001).The comparative response of five *Brachiaria* species under controlled conditions has been studied by Guenni *et al*, (2002) and got similar results. Based on analysis of 35 chickpea cultivars, Talebi *et al.*,(2013) concluded that there was substantial variation in tolerance to drought within chickpea cultivars. Drought-tolerant cultivars had higher RWC, chlorophyll, carotenoid and Na^+ and K^+ uptake. The protective role of K by maintaining high pH in stroma and controlling photooxidative damage in plants suffering from drought stress was reported by Cakmak (1997). Under water deficit conditions, K nutrition increases crop tolerance by utilizing the soil moisture more efficiently than in K deficient plants.

On the contrary, Water shortage reduced univalent K^+ cations concentrations in the petioles of *Beta vulgaris* (Choluj *et al.*,2008). Potassium concentrations were decreased upto 94% in roots of *Fagus sylvatica* L**.** due to drought (Peuke and Rennenberg, 2011). Under drought conditions, chloroplast loose high amounts of K to depress photosynthesis further (Sen gupta and Berkowotz, 1987) and induce ROS formation. This strongly supports the idea that increase in severity of drought stress results in corresponding increase in K demand to maintain photosynthesis and protect chloroplast from oxidative damage.

Similar observations were made by Rao and Ramamoorthy, (1981) in *Vigna mungo, Vigna unguiculata* and *Glycine max.* and indicated that K uptake is only slightly reduced by water stress. Farah (1981) reported reduction in the amount of leaf potassium in beans throughout the growth period of bean. Kaya *et al.*, (2006) also reported a reduction in potassium in maize leaves under drought condition. Nikam (2007) observed that K content is greatly reduced in leaf tissue of *Chlorophytum borivilianum* due to water stress. Due to one month long term water stress in leaves of *Gongronema latifolium* there was significant reduction in potassium content. This was the result of mobilization of these minerals from the leaves to the roots of stressed plants where they may act as osmoprotectant assisting the plants to resist water stress (Osuagwu and Edegoa, 2012).

From the present findings, it can be seen that with increasing water stress there is increase in K content, in *Aristolochia bracteolata* Lam. The enhancement is significant in shoot part especially in stem. Accumulation of potassium in shoot part is accounted for osmotic adjustment and this may be helping the regulation of metabolism and transport under unfavourable conditions.

4. **Calcium** :

In Table 29, effect of water stress on calcium content in leaf, stem and roots of *Aristolochia bracteolata* Lam. is presented. Highest calcium content is recorded in leaf (71.61 mg/g) followed by stem (31.10 mg/g) and root (17.72 mg/g). The stress showed decline in calcium content in leaf followed by stem while roots show very small increment. As per the Table, it is interesting to note that in leaf there is higher desiccation in calcium in leaf, with increasing water deficit (72 hrs) there is decrease in calcium content from 716.10 to 311.89 mg/g. as compared to stem 31.10 mg/g to 26.2 mg/g). Quite a different trend of the uptake and distribution of this divalent cation is observed in root. It accumulates from 17.72 mg/g to 17.97 mg/g in roots. It is obvious from the figures that the concentrations of calcium in leaf, stem and root are varied with water stress.

Calcium (Ca) is an essential plant macronutrient with key structural and signalling roles. Calcium ions (Ca^{2+}) act as an osmoticum within vacuoles; a stabilizing element of membranes; a strengthening agent in cell walls; and a secondary messenger for a multitude of signals (Dodd *et al.*,2010). It has a very prominent role in maintenance of cell structure. It activates the plasma membrane enzyme ATPase which pumps back the nutrients lost during cell membrane damage due to Ca deficiency and recovers plant from injury (Waraich *et al.,* 2011). Ca and biofortification are both active fields of research and manipulation of either entity in isolation may have a negative impact on the other and eventually on the overall productivity of targeted food crops (Dayod *et al.*,2010). From discovery of calmodulin, it has become clear that Ca is not just a macronutrient but also a major controller of plant metabolism and development (Poovaiah and Reddy, 2000). Ca is necessary for recovery from drought by activating the plasma membrane. Number of experiments has been made to study the effect of water deficit on calcium nutrition of plants to study its key role in plant metabolism.

Nigwekar (1988) observed that the calcium content increased in the leaf and stem of horse gram due to water stress. Similarly Sadale (2007) observed that with the exception of 1 and 2 day water stress in stem and 4 days water stress in nodules the concentration of calcium was increased in all parts of *Sesbania grandiflora* under water stress condition.

Whereas in sugar beet water shortage reduced Ca^{2+} ion level in the mature and old leaves of sugarbeet (Choluj *et al.,* 2008). It has been suggested that the decrease in calcium content in the leaves of *G.latifolium* might be result of it's mobilization from leaves to the roots under stress where it acts as osmoprotectant, assisting the plant to resist drought (Osuagwu and Edeoga, 2012).

In *Aristolochia bracteolata* Lam. also similar decline in Ca content in leaf and stem tissue and its accumulation in roots is recorded. This mobilization may be helpful to overcome drought stress as it may be acting as osmoprotectant at root level.

5. Magnesium :

Magnesium content in leaf, stem and roots of *Aristolochia bracteolata* Lam. is presented in Table 29. It also depicts the effect of water stress on magnesium content in these parts. In *Aristolochia bracteolata* Lam. highest Mg content is recorded in leaf (20.57 mg/g) followed by root (15.23 mg/g) and stem (13.58 mg/g). Water stress showed meagre rise in magnesium content in leaf followed by stem while in roots there is very small decline. As per the Table, in leaf and stem samples with increasing water deficit (72 hrs) again there is overall accumulation trend of magnesium content i.e. leaf from 20.57 to 21.39 mg/g and in stem 13.58 to 18.93 mg/g. Among the various plant parts, however, the increase in magnesium is quite significant in stem followed by leaf. Whereas in root decline in Mg content was from 15.23 to 14.80 mg/g.

Magnesium is an important, mobile and strongly electropositive divalent cation in the plants. It plays a crucial role in various physiological processes. The most well known role of Mg is its contribution to the centre of the chlorophyll molecule, although chlorophyll Mg is relatively in small fraction of the total Mg content of a plant (Mengel and Kirkby, 2001). It is essential for the transfer of phosphate group from ATP, proper functioning of some enzymes related with energy transfer and necessary for muscular contraction contraction (Oram, 1983). It also

regulates the plant metabolism by acting as cofactor or activator of enzymes, and as a component of chlorophyll.

A study by Hu and Schmidhalter (1997) revealed that the Mg^{2+} concentration in wheat leaves was reduced, the biomass and Mg concentration decreased with water stress in all the wheat genotype along wih Ca and P. While Huang (2001) observed decrease in Magnesium concentration in shoot of drought stressed plants of all cultivars of tall fescue. In 2007, Sadale observed decrease in Mg content in all plant parts like root, stem and leaves in *Sesbania grandiflora* with short term water stress but there was negative correlation between Mg concentration and level of water stress. While long-term drought stress reduced Mg^{2+} ions level in the mature and old leaves of sugar beet was observed by Choluj *et al.*,(2008).

On the other hand, Water stress generally favoured the increase of Mg as the relative amounts of Mg increased considerably more in barley than in rye when water stresses were imposed (Rahman *et al.*, 1971). Wang and Gao (2004) observed that alongwith Mg concentration in root, stem and leaf of bald cypreus increased with decreasing soil water contents and more accumulation was observed in leaf than root and stem. Nikam (2007) observed that level of magnesium in both leaves and tubers of *Chlorophytum* was slightly elevated and concluded that it may help in maintenance of metabolism rather than causing any adverse effect. Drought had no significant effect on the percentage magnesium content of the leaves of *Gongronema latifolium* (Osuagwu and Edeoga, 2012).

From the obtained results it is observed that Mg content has shown increment in both leaf and stem and slight decrease in root tissue. Retention of chlorophylls in the leaves under drought stress in this plant also supports these findings. Role of Mg as cofactor for various enzymes related with energy transfer is well known. The high Mg content in water stressed *Aristolochia* points towards plants adaptive response against stress. Several reports also justify our findings.

6. Sulphur :

Sulphur content in leaf, stem and roots of *Aristolochia bracteolata* Lam. is presented in Table 29. The same Table also depicts the effect of water stress on sulphur content in these parts. In *Aristolochia bracteolata* Lam. highest Sulphur content is recorded in leaf (71.73 mg/g) followed by stem (40.54 mg/g) and root

(34.30 mg/g). Thus in this plant leaf showed highest sulphur content indicating it's accumulation and metabolic role. The water stress showed meagre rise in sulphur content in leaf followed by stem and roots. As per the Table, it is interesting to note in leaf, stem and root with increasing water deficit (72 hrs) again there is overall trend of accumulation sulphur content i.e. leaf from 71.73 to 87.32 mg/g, in stem 40.54 to 71.73 mg/g followed by root 34.30 to 56.13 mg/g. Among the various plant parts, however, the increase in sulphur is quite significant in stem followed by root and leaf.

It is evident from the Table 29 that water stress positively affected sulphar metabolism but more pronounced effect was found in stem followed by root and then in leaf. In stem it increased from 40.54 to 91.73 mg /gm dry matter.

There are many S-containing compounds, which have been linked, directly or indirectly with the defence of plants against microbial pathogens; these include thionins, defensins, glucosinolates, crucifer phytoalexins and glutathione (Hell, 1997). Plants contain a variety of sulphate transporters with specific functions in the uptake of sulphate by the roots, its transport to the shoot and its subcellular distribution (Hawkesford *et al.,* 2003). Sulphur is also required for the synthesis of various other compounds, such as thiols (glutathione), sulpholipids and secondary sulphur compounds (glucosinolates, phytochelatins) which play an important role in plant metabolism, in the protection and adaptation of plants against stress and pests. According to De Kok *et al.*(2002) sulphur is so essential that its deficiency results in the loss of plants resistance to environmental stress and pests.

Utrillas *et al.,* (1995) noticed that in summer months the concentration of sulphur in *Cyanodon dactylon* was reduced when subjected to water stress. Nikam (2007) also observed that in *Chlorophytum borivilianum*, sulphur level in tuber slightly showed decrease in short term water stress while in long term water stress the level of this element showed marked increase.

Wallin *et al.*(2002) reported that in the needles of spruce samplings after drought treatments significant increase in Sulphur was found.

The effect of moisture stress on the uptake of sulphur in *Sorghum vulgare* plants studied by Freney *et al*., (1975). And they observed that sulphur content had been stimulated and little effect had been noticed. They concluded that soil organic matter fractions as sources of plant available Sulphur. Gooding *et al.,* (2003)

observed that restricted water availability on the winter wheat grains had no effect on N: S ratio.

From our results, it is well evident that with increasing water stress there is an increase in the level of S in leaf and stem of *Aristolochia bracteolata* Lam. The increment may be attributed towards protective role of sulphur under water stress. Further, our findings of aristolochic acid increment under stress condition also justify the same.

7. Sodium :

Effect of water stress on sodium content in leaf, stem and roots of *Aristolochia bracteolata* Lam. is presented in Table 29. Highest Na content is recorded in leaf (86.96 mg/g) followed by stem (56.53 mg/g) and root (25.65 mg/g). The water stress showed rise in sodium content in stem but significant decline in leaf followed by roots. As per the Table, it is to note that in with increasing water deficit (72 hrs) there is accumulation of sodium content in stem from 56.53 to 59.57 mg/g while in leaf it declined from 86.96 to 50.00 mg/g followed by root 25.65 to 21.74 mg/g. Among the various plant parts, however, accumulation of sodium is more in shoot tissue that stem reflecting its moderate drought tolerance capacity.

Sodium is one of the trace elements involved in K antagonism. It is well established that sodium is an essential micronutrient for C_4 photosynthesis in C_4 plants (Brownwell and Crossland, 1972). Na is accumulated passively in plants and it always interferes with metabolically active K. This monovalent cation plays significant role in halophytes (Popp, 1983). Role of sodium in maintaining favourable water balance has been suggested by Rains (1972). According to Brownwell (1979) sodium may play a role in preventing leakage of carbon compounds from the metabolic system of plant.

The sodium concentration in a leaf and its mobilization was studied under various parameters like, transpiration rate, growth rate as well as total volume of water already transpired during life span of the leaf. Comparison with Na in xylem and phloem revealed that very small fraction of Na is remobilised in the phloem (Munns, 2005). Sodium contributes to osmotic potential maintains favorable water balance and increases the drought resistance to plants.

Its uptake and distribution in the plants has rarely been investigated in plants grown under water stressed conditions. According to Lawlor and Milford (1973) in sugarbeet sodium content increases the drought resistance by altering leaf water balance under water stress condition. Longer duration of water stress in *C. borivillianum* has caused slight increase in sodium content in leaf tissue is observed by Nikam, (2007). Similarly work of Jadhav (2000) in Prosomillet showed that an efficient potassium and sodium uptake can improve water balance of this plant under stress condition. Zhu *et al.,* (2005) reported that there is accumulation of Na in Dingxi 24 drought tolerant variety more than cv. Longchun 8139 drought sensitive wheat variety. Sadale (2007) also observed that in *Sesbania grandiflora* sodium concentration increased due to water stress.

There are also reports of decline in Na content under water stress. In *Cynadon dactylon,* a decrease in Sodium content (71%) under water deficit was recorded by Utrillas *et al.,*(1995) and in alfalfa by Goicoechaea *et al*.(1997) in Bald cypress by Wang and Gao (2004). Influence of long-term drought stress on osmolyte accumulation in sugarbeet reduced univalent (K^+, Na^+) cation concentrations in the petioles of sugarbeet (Chołuj *et al.,* 2008). Delfín *et al.,* (2012) observed that Na uptake increased in two sweet potato cultivars under stress conditions.

While Ma *et al.,* (2004) reported that Na did not contribute significantly to osmotic adjustment and solute accumulation in comparison with other ions in leaves of *Brassica* in response to soil water deficit. While Osuagwu and Edeoga (2012) observed that water stress has no significant effect on the percentage concentration of sodium of the leaves of *Gongronem latifolium*.

The present findings *Aristolochia bracteolata* Lam. showed sodium content higher in leaves followed by stem and roots. With increasing water stress there is overall decline in Na content and this effect is more profound in leaves as well as roots. Lowering of Na content coincides with increased K content under stress thereby enhancing the capability of the plant to sustain under water deficit and able to maintain higher content Na in leaf tissue but not in stem and root tissue.

8. Iron :

It can be revealed fromt Table 29 that iron content is more in roots (13.57 mg/g) than stem (12.89 mg/g) and leaf tissue (12.53 mg/g). Further, with water stress

slight rise in iron content is seen in all root, stem, and leaf tissue of *A. bracteolata* Lam. (Table 29). However, the rise in iron content is not substantial and the values are meagre in roots than stem and leaf.

Though considered as microelement, Fe is very essential for enzyme systems where it performs heem or haemin function as prosthetic group. It has a specific role in chlorophyll synthesis, because iron-porphyrin compound is formed as an intermediate of chlorophyll synthesis. Iron is also reported to be directly implicated in nucleic acid metabolism and also plays a regulatory role in nitrogen fixation and in nitrate reduction in plants (Price *et al.*,1989). Iron is involved in photooxidation reduction reactions via ferredoxin as well as in chlorophyll synthesis (Spiller and Terry, 1980). Iron is a constituent of enzymes like POX, CAT, cytochrome oxidase etc. and is required for activation of number of other enzymes.

There are many attempts made to study influence of drought on iron nutrition. Decrease in iron content due to water stress has been recorded in *Medicago sativa* (Goicoechea *et al.*,1997). Wang and Gao (2004) studied the effect of soil water content on nutrient uptake and allocation in *Taxadium distichum* and reported that total accumulation of nutrient including iron decreased with decreasing soil water content. Molassiotis *et al.* (2006) reported Fe decrease in response to water stress in apple.

On the contrary, an increase in iron content has been recorded in pearlmillet due to water stress by Chavan (1980) and in horsegram by Nigwekar (1988). Increase in iron concentration of roots of water stressed apple plant was reported by Ivanov and Karakash (1965). Kkuzmina & Frshov (1999) observed iron accumulation in durum wheat leaves under drought conditions This may be leading to metabolic disorder resulting decrease in productivity. Al Karkui (1998) reported similar results in wheat plants by water stress Fe content was higher in shoots and in CR006 genotype of wheat, enhancement of Fe uptake due to AM inoculate was more pronounced particularly under water stress. Similarly, Sadale (2000) in suggested that *Sesbania grandiflora* iron concentration increased in root and stem with decrease in leaves due to Fe translocation from stem to leaves when the plants were subjected to water stress. Nikam (2007) reported more pronounced accumulation of iron in tuber tissue than leaves of *Chlorophytum borivilianum* . He suggested that this may have lead to iron toxicity under water stress.

From the results obtained with *A. bracteolata* Lam. both uptake and translocation of iron is stimulated by water stress as there is higher accumulation of Fe in roots than shoot part i.e.stem and leaf.

9. Manganese : Manganese content in leaf, stem and roots of *Aristolochia bracteolata* Lam. is presented in Table 29. The same Table also depicts the effect of water stress on Manganese content in these parts. In *Aristolochia bracteolata* Lam. highest Mn^{2+} content is recorded in leaf (0.95 mg/g) followed by stem (0.73 mg/g) and root (0.56 mg/g). The water stress showed significant rise in manganese content in root meagre increase and in stem it declined from 0.73 mg/g to 0.42 mg/g. As per the Table, with increasing water deficit (72 hrs) there is accumulation of manganese content in root from 0.56 to 1.46 mg/g and in leaf it increases from 0.95 to 0.96 mg/g stem.

Manganese constitutes an important micronutrient in plants which is involved in several enzyme systems as a cofactor especially in oxidation reduction reactions of carbohydrates and IAA. This element is involved in regulating the decarboxylating enzymes of C_4 pathway, especially NADP- malic enzyme, NAD malic enzyme and PEP carboxykinase which is highly Mn^{2+} specific. Photosystem II is believed to contain manganese protein which catalyses the early stages of O_2 evolution (Basiounty and Biggs, 1976). It is well known that there are about 5 -8 atoms of Mn per 400 molecules of chlorophyll.

Zerki (1995), reported higher concentration of Mn in leaves of citrus cultivars subjected to PEG induced water stress. Under water stress condition Mn^{2+} content is increased in the roots and leaves of *Sesbania grandiflora* (Sadale, 2000), in seeds of soyabean (Samarah *et al.*, 2004) and they attributed that this as response to drought stress tolerance.

On the contrary, experiment of Pande and Singh (1969) revealed reduction in Mn content in rice plants under stress. Maksimovic (2003) studied the effect of water stress on accumulation of micronutrients in eight Sugerbeet genotype leaves and observed that there was overall decline in Mn content along with Cu, Zn and Fe, and concluded that expression of genes coding for Mn containing SOD enzymes can be induced by the lack of water or by rehydration of plants which had experienced drought. Increase in manganese content was observed in varieties of *Piper nigrum* L., Uthirankotta-2,

Kumbakodi and Panniyur-5, but severe stress decreased manganese content in all the varieties except Poonjarmunda variety Thankmani and Ashokan (2004). Rahdari and Hoseini, (2012) observed that there was meaningful decrease in manganese content synchronized with increasing of stress induced by PEG in *Portulaca oleraceae* L. leaves.

It is well evident from the results that there is overall decline in Mn content in shoot part i.e. leaf and stem, while an increment is seen in root under increasing water stress in *Aristolochia bracteolata* Lam. Present findings clearly indicated mobilisation of Mn from shoot part towards roots. As Mn^{2+} is known for its role in oxidative enzymes as well as in proteolytic enzymes, the Mn accumulation and its transport suggests stimulation of osmoregulation and ROS activity under water deficit.

10. Zinc:

Effect of water stress on Zinc content in leaf, stem and roots of *Aristolochia bracteolata* Lam. is presented in Table 29. Highest Zn^{2+} content is recorded in leaf (4.47 mg/g) followed by stem (1.76 mg/g) and root (0.80 mg/g). Thus leaf showed highest Zinc content indicating it's accumulation. The water stress showed significant rise in Zinc content in root and in stem while decline in leaf tissue. As per the Table, it is interesting to note in leaf, stem and root samples with increasing water deficit (72 hrs) there is accumulation trend of Zinc content in root from 0.80 to 3.21 mg/g and in stem from 1.76 to 2.43 mg/g whereas decrease in zinc content in leaf tissue from 4.47 mg/g to 1.54 mg/g. Among the various plant parts, however, accumulation of Zinc more in root tissue than shoot parts.

Zn is an important micronutrient as it improves root growth which in turn enhances the intake of water which helps in stomatal regulation and enhances the drought tolerance (Waraich *et al.,*2011). Zn is highly mobile in the soil, easily taken up by plants, and can thus accumulate to extremely high phytotoxic concentrations in the biomass (Vysloužilová *et al*., 2003). The accumulation rate depends on soil conditions, plant species, genotype, climate, and agronomic practice (Tlustos *et al*., 2001).

There are many reports on effect of drought on Zn content in plant. Zubaidi *et al.,* (1999) observed that number of genotypes of durum wheat had maximum Zn accumulation up to twice, as compared to other genotypes grown under drought condition. Accumulation of Zinc in leaves and seeds of cowpea was also observed by Ei-Bassiouny and Shurky (2001). However, the concentrations of Zn increased in the seeds of Soyabean under drought condition reported by (Samarah *et al.*, 2004), in wheet shoots by Al-karaki *et al.,* (1998), in citrus fruits by Zerki (1995). Zinc content of the leaves increased due to water stress in Panniyur-5 variety of Piper *nigrum* L. by Thankmani and Ashokan in 2004. Marginal increase in Zn level in shoots and reduction in Zn level in roots response to water stress in *Chlorophytum borivillinum.* (Nikam, 2007). While Sadale (2007) reported significant increase in Zn concentration in all parts of *S.Grandiflora.* Whereas in cherry rootstock Gisela water deficit did not significantly influence Zn content (Sivritepe *et al*., 2008).

On the other hand, Goicoechea *et al.,* (1997) observed decrease in Zn concentration in alfalfa due to water stress. Pirzad *et al.,* (2012) observed that excess water reduced Zn uptake in *Matricaria chamomilla* L. plants.

It is well evident from the Table that with increasing water stress Zn has been mobilized from leaf and stem i.e. shoot part and accumulated in roots of *Aristolochia bracteolata* Lam. As Zn is regulating water uptake mechanism it may be repairing the membrane damage caused in roots under water deficit. This can be treated as an adaptive feature, also playing as a protecting ROS activity.

11. **Copper** :

Copper content in leaf, stem and roots of *Aristolochia bracteolata* Lam. are presented in Table 29. The same Table also depicts the effect of water stress on Copper content in these parts. In *Aristolochia bracteolata* Lam. highest copper content is recorded in leaf (0.61 mg/g) followed by stem (0.36 mg/g) and root (0.27 mg/g). Water stress caused rise in Copper content in only roots but this rise is not much significant while decline in leaf as well as stem tissue. As per the Table, with increasing water deficit (72 hrs) there is accumulation of Copper content in only in root from 0.27 to 0.47 mg/g while in leaf and stem it has shown decrease.

Copper is a constituent of the chloroplast protein Plastocyanin which forms a part of the electron transport chain linking the two photochemical systems of

photosynthesis. Cu is known to regulate the plant metabolism even under stress conditions by acting as component of lactase, cytochrome C oxidase, Ascorbic acid oxidase and polyphenol oxidase (Nicholas, 1975). Also, this is a key micronutrient in defence mechanism of plants under abiotic stress. Cu alleviate the adverse effect of drought indirectly by activating the physiological biochemical and metabolic processes in plants (Waraich *et al.*,2011).

There is least work on fate of this divalent cation, a metallic ion in plants under stress conditions. Very few of them like Salunkhe (1990) observed an increase in copper content in root of *Dodonea viscosa* L. as there was substantial increase in Cu content. Al-karaki and Clark (1998) noticed higher concentration of Cu in shoot of water stressed wheat plants. Copper content in the leaves increased due to water stress in Panniyur-5 variety of Piper *nigrum* L. (Thankmani and Ashokan, 2004). Similarly, Samarah (2004) also observed increased concentration of Cu in Soyabean seeds in response to water stress. Goicoechea *et al.*,(1997) observed enhancement of Cu levels in alfalfa plants in response to drought as well as in cowpea by Ei Bassiouny and Surky (2001).

However, Sadale (2002) observed Cu is accumulation in roots and nodules of *Sesbania grandiflora* preliminary in water stress but there after decreased while increased in stem. Whereas Nikam (2007) observed that in *C. borivillinum* due to water stress Cu content lowered in leaves and accumulated in tubers.

The contents of Cu in the leaves of young plants of all eight sugarbeet genotypes declined under water deficiency conditions as observed by Maksimovic (2003) and he suggested that Cu is components of superoxide dismutase (SOD), which exists in two forms: Cu /Zn SOD. It was found that the expression of genes coding for this enzymes could be induced by the lack of water.

It is well evident from the Table 29 that due to water stress there is increment in Cu content in roots while it declined in shoot part i.e. leaf and stem. As Cu is involved in ROS activity through various oxidases, in present activity in roots and this may be against osmoregulation.

It seems that due to water deficit Cu is mobilized from shoot part to roots and this may be a strategy of drought tolerance through osmoprotection. It is interesting that due to water deficit all oxidases in leaves have shown significant increment in activity indicating activated drought tolerance mechanism in this weed.

c) Organic Solutes Under Water stress

1) Total protein and Soluble Protein

The changes in level of total proteins and soluble proteins in leaf, stem, and root of *Aristolochia bracteolata* Lam. due to water stress are presented in Table 30 and 31 respectively. In *Aristolochia* highest soluble protein content is recorded in leaf (15.54 mg/g), followed by stem (11.89 mg/g) and root (8.72 mg/g). It is evident that in this plant highest level of soluble proteins in leaf indicating it's accumulation and utilization. The water stress showed significant decline in protein content in leaf, followed by stem while in the roots shows marginal decrease. As per the results, it is evident that with water stress the general trend of protein accumulation in different plant parts is the same. i.e. leaf protein content has highest values while the lowest one's are recorded in roots. The decrease is more pronounced in leaf and shoot, than roots, when compared with moisture percentage it is quite clear that with increasing water deficit soluble protein content is lowering significantly. Whereas, on the contrary, at all water stress levels total proteins increased in leaf, stem and root tissues of *Aristolochia bracteolata* Lam.

It seems that the initial increase in total soluble proteins during drought stress was due to the expression of new stress proteins, but the decrease was due to a severe decrease in photosynthesis as photosynthesis decreased in drought stress. There are several reports of inhibition of protein synthesis under water stress conditions.

Early in 1974, Kramer reported that the first measurable effect due to water deficit was growth reduction caused by declining cellular expansion. While Costa *et al.,* (2008) reported decrease in amount of total proteins because of decrease in their synthesis and and also fall in nitrate reductase activity in two *Vigna ugulculata* Walp. cultivates under water stress. Bayramov,(2010) reported that at the early stage of heading, during 2-3 days of water stress, protein content of PRK, FBP and NADP-GAPDH was unchanged in flag leaves, while in ear elements protein content of these enzymes decreased significantly in the wheat genotype Garagylchyg-2.

Changes in protein expression, accumulation and synthesis have been observed in tomato plant due to drought stress by Tabaeizadeh *et al.,* (1995) and in *Sorghum* (Cheng *et al.,*1993). Both quantitative and qualitative changes to proteins

Table 30 Effect of water stress on Total proteins in *Aristolochia bracteolata* Lam.

Plant Part	Total Proteins mg / g dry tissue			
	a	b	c	d
Leaf	310.25	301.99	384.60	350.64
Stem	141.36	119.33	155.13	186.34
Root	102.81	111.07	100.97	133.10

Table 31 -Effect of water stress on Soluble Protein content in *A. bracteolata* Lam.

Plant Part	Soluble Proteins mg/ g fresh tissue			
	a	b	c	d
Leaf	15.45 ± 0.01	13.47 ± 0.05*	12.68 ± 0.05**	7.92 ± 0.01**
Stem	11.89 ± 0.01	10.50 ± 0.05**	9.91 ± 0.01**	6.14 ± 0.02**
Root	8.72 ± 0.03	7.13 ± 0.01**	6.14 ± 0.02**	3.77 ± 0.26**

(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1 days no water

(c) 48 hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with 3 days no water

* values are significant at 5 % level where $P < 0.05$.

** values are non- significant at 5 % level

were studied during drought stress by Ricardi *et al.,* (1998) in maize plant. It was observed that there was an increase in expression of 50 proteins, decrease in 23 and induced synthesis of 10 proteins. The process of cellular elongation and carbohydrate wall synthesis were very susceptible to water deficit, and the growth decrease was a consequence of the turgescence laying down those cells (Kramer and Boyer, 1995).

Under stress condition plant synthesizes heat shock proteins. The essential function of heat shock proteins is to prevent aggregation and assist refolding of non-active proteins under both normal and stress conditions (Frydman, 2001). According to Mohammad khani and Heidari (2008) there was decrease in total proteins content in roots but an increase in leaves of two maize varieties CV 301 and CV 704. The accumulation of dehydrin like proteins was detected in the roots and leaves of drought-stressed two varieties of maize, suggesting this could protect plants from further dehydration damage. Bradford and Chandeler, (1992) proposed that proteins synthesized in response to water stress are called dehydrins and studied expression of Proteins in Embryos and Seedlings of *Zizania palustris* and *Oryza sativa* during Water stress.They observed that Seeds of *Zizania palustris* are tolerant of dehydration to <10% moisture content on fresh weight basis) while seeds of *Oryza sativa* L. readily tolerate desiccation to <5% water content. Ali and Basha (1998) showed that total protein content of peanut leaves significantly increased when plants were subjected to water stress for 5 to 20 days as compared to irrigated controls. Drought stress induced changes in protein synthesis in maize.

Similarly Pinheiro *et al.,* (2001) showed that water deficit in *L. albus* brings about induction of thraumatin like protein (TL). Soluble proteins, sugars and free amino acids including proline accumulated significantly in response to drought stress in two *Vicia faba* cultivars (EI-Tayeb, 2006) observed level of soluble proteins in nodules increased after one and two day water stress and decreased after four day water stress (Sadale, 2007). Beneficial effects on growth and total soluble proteins in *Lupin albus* plant was also observed under water stress. (Abdalla *et al.,* 2011). In his study diatomite application either alone or combined with stress induced a distinctive protein electrophoratic band of 92KD. Three distinctive protein bands were also induced in stressed white lupin, two (65 and 25 KD) under one day drought and one (143K) under four day drought. Drought stress significantly affected proteins in *Vitex trifolia* L., *Vitex altissima* L. and *Vitex negundo* L. plants when compared with normal. (John *et al,* 2011). They reported several new protein bands in stressed plants.

It seems that *Vitex* species can be adapted to drought stress conditions by synthesizing new proteins in stressed plants for their adaptation and these proteins could be used as markers in identifying the stressed plants.

Thus, in the present findings reduction in soluble proteins in *Aristolochia bracteolata* Lam. due to water stress is recorded. This can be attributed to increased proteolytic and oxidative enzymes with increasing water deficit. Also, the results indicate that the normal protein profile is altered and new drought induced proteins may have been synthesized to adapt this species under drought.

2) Carbohydrates:

The effect of Water stress carbohydrates in *Aristolochia bracteolata* Lam. is depicted in Table 32. It is clear from the Table that level of carbohydrates in all parts shows decline but marked decline was found in root tissue. In leaf, after 24 hrs carbohydrate content increased from 19.44 mg/g to 19.90 mg/g but afterwards decrease to 18.53 mg/g at the end of water stress treatment. While in stem it decreases from 18.33 mg/g to 16.71 mg/g and in roots it declines from 17.23 mg/g to 14.94 mg/g. This represents a gradual fall in the overall metabolic order of plant.

Carbohydrates provide carbon skeleton for wide range of carbon compounds present in the plant tissue. Change in its metabolism is of particular importance on account of their direct relationships with such physiological processes as photosynthesis, translocation and respiration. According to Parker (1956) sugar content may result in a decrease in transpiration.

Water stress affects many physiological and biochemical processes in plants involving carbohydrates metabolism with accumulation of sugars and number of organic solutes (Kameli, 1990).

Dina and Klickoff (1973) studied effect of plant moisture stress on carbohydrate on big sagebrush and concluded that Sugar increases in the leaves, stem, and roots of big sagebrush due to of water stress may be of adaptive significance. In 1992, Kameli and Losel studied changes in sugar content during water stress in leaves of two varieties *Triticum durum.* They observed that glucose and fructose accumulated in leaves of wheat and concluded that contributions of sugars and other solutes to osmotic adjustment and studies on the partitioning of photosynthates in carbohydrate.

Table 32- Effect of water stress on total Carbohydrates in *A. bracteolata* Lam

Plant Part	Total Carbohydrates (mg/100 g fresh tissue)			
	a	b	c	d
Leaf	19.44 ± 0.03	19.90 ± 0.06*	18.34 ± 0.01**	18.53 ± 0.01**
Stem	18.33 ± 0.06	17.68 ± 0.01**	17.03 ± 0.72	16.71 ± 0.02**
Root	17.23 ± 0.28	16.31 ± 0.01*	15.79 ± 0.02**	14.94 ±0.57*

(a) Control – Plants in pots watered everyday.

(b) 24hrs –Plants in pots with 1 days no water

(c) 48hrs - Plants in pots with 2 days no water

(d) 72hrs -Plants in pots with 3 days no water

* - Values are significant at 5% level where $P < 0.05$.

** values are non- significant at 5 % level

Whereas Epron and Dreyer (1996) worked on Starch and Soluble carbohydrates in leaves of water stressed oak saplings they observed overall accumulation of glucose and fructose while decline in sucrose and starch concluded that drought induced changes in sugar contribute to osmotic adjustment**.**

According to Ackerson, (1981) increase in leaf carbohydrate helps in osmotic adjustment due to water stress in cotton. Studies conducted on peas (Ford, 1984), tomato (Handa *et al.,* 1983), pigeon pea (Keller and Ludlow, 1993) and corn (Premchandra *et al.,* 1993) showed a common trend of increased soluble sugars as a result of drought stress.

Timpa *et al.,* (1986) observed the water stressed cotton plants showed 2-5 times greater amount of organic acids and carbohydrates over the irrigated ones. Silvreira *et al.* (2003) in cowpea plants showed stressed nodules had significant accumulation of soluble carbohydrates. While poor recover of cowpea nodules after rehydration was apparently related to a carbohydrate shortage.

It seems that concurrent with the induction of drought stress or reduction of soil water content and with accumulation of soluble carbohydrates, protective enzymes increased in plants under stress (Li *et al.,* 2010).

Arabzadeh (2012), observed that drought stress affected soluble carbohydrates in two species of *Haloxylon persicum* and *H. aphyllum* as there was an increase in the rate of soluble sugars in branches and roots of both species were in proportion with stress. While Pirzad *et al.,* (2011) observed that there were not any changes in amount of total soluble carbohydrates at the experimental range of water stress in *Matricana chamomilla* L.

While, on the contrary, Wang *et al*.,(1995) while studying effect of water stress in apple reported that the percentage of soluble carbohydrate sorbitol decreased in roots where as sucrose decreased in mature leaves and stem, however increased in young leaves and roots and differential changes in root and shoot carbohydrate was observed by increasing stress.

In present work reduction in carbohydrates in all parts of *Aristolochia bracteolata* Lam. under water stress confirms the prevention from cellular damage as short term drought has a negative correlation with decrease in quantities of

carbohydrates. The decrease in total carbohydrates may be accounted for alteration of metabolic pathways leading to adaptive trend of survival under water deficit.

d. Enzyme studies under water stress

1. Peroxidase :

The activity of enzyme peroxidase in leaves of *A. bracteolata* Lam. is recorded in Table 33 and figure 23. It is obvious from the results that the activity of this enzyme in leaf is increased with water stress. The activity of peroxidase in leaf is about more than double (29.50 Δ O.D. h^{-1} mg^{-1} protein) as compared to control (12.00 Δ O. D.h^{-1} mg^{-1} protein).

Free oxygen radicals, produced as the usual secondary consequence of enviormental stresses are very dangerous for all cell components and must be precisely regulated. All plants have developed several antioxidant systems as catalase, superoxide dismutase, peroxidase, glutathione reductase are prominent.

Drought induces oxidative stress in plants, in which ROS such as Superoxide radical (O^{2}), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and alkoxy radical (RO) are produced (Munn-Bosch and Penuelas, 2004, Esfandiari *et al.*, 2008). PODS are often the first enzymes which are altered under drought. An increase in POD could act as an efficient H_2O_2 scavenging system in plant vacuoles in the presence of phenolics and reduced ascorbates, phenolics are oxidized to phenoxyl radicles which can be reduced by ascorbate (Zivkovic *et al.*,2010).

Chakraborty *et al.,* (2001) reported that peroxidase activity initially increased in leaves of tea plants while decreased during extended drought period. Deshmukh and Dhumal (2005) also reported peroxidase activity stimulated with increasing water stress in *Sorghum* cultivars and concluded that enzymes like peroxidase, polyphenol oxidase and NR act as the indicators for testing the water stress tolerance and might be useful for *Sorghum* breeder to evolve drought to tolerant cultivar.

However, involvement of antioxidants and lipid peroxidation in the adaptation of two cool- season grasses to localized drought stress peroxidase activities remained unchanged during most period of surface drying (Jinmin and Bingru, 2001).

Table 33 –Effect of water stress on different oxidase activities of *A. bracteolata* Lam. Leaves.

Enzyme	a	b	c	d
Peroxidase (Δ O.D.min^{-1} gm^{-1})	12.00±0.15	24.50±0.12*	25.50±0.4*	29.50±0.3*
Catalase (mg H_2O_2 liberated $min^{-1}g^{-1}$)	1.45 ±0.02	1.70 ±0.02*	1.81 ±0.03*	2.04±0.03*
IAA Oxidase (Δ O.D.h^{-1} mg^{-1})	0.22 ±0.02	0.56±0.01*	0.57 ±0.02*	0.66 ±0.04*
Polyphenol oxidase (Δ O.D.$min^{-1}g^{-1}$)	0.56 ±0.01	0.60 ±0.01*	0.65 ±0.02*	0.95 ±0.01*

(a) Control – Plants in pots watered everyday.

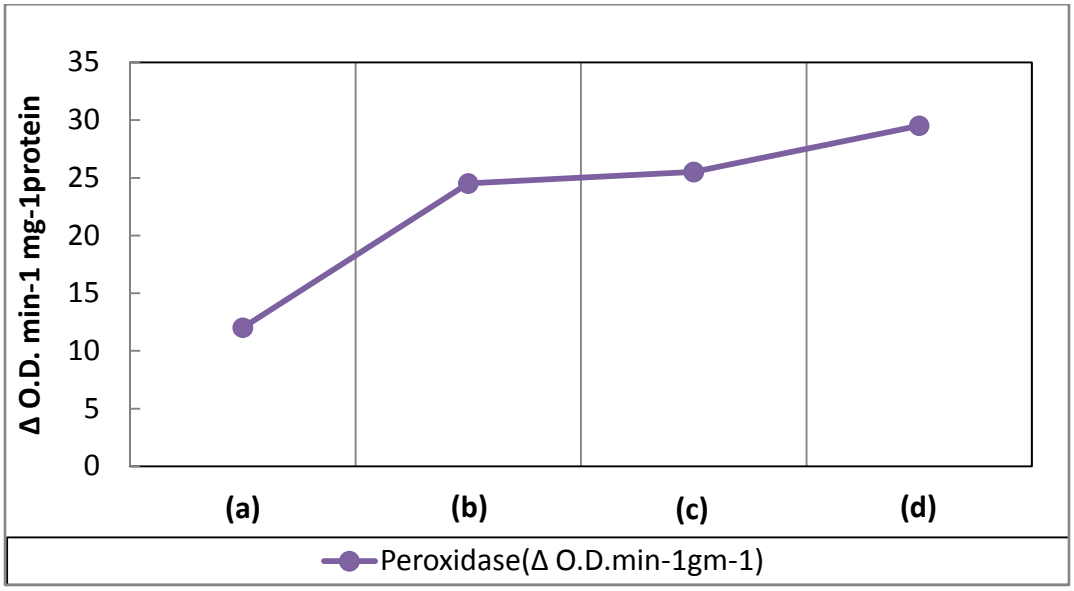
(b) 24 hrs –Plants in pots with 1 days no water

(c) 48 hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with 3 days no water

* - Values are significant at 5% level where $P < 0.05$.

Figure 23 Effect of Water Stress on peroxidase activity in leaves of *Aristolochia bracteolata* Lam.



(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1 days no water

(c) 48 hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with 3 days no water

Nikam (2002) in his study on *Chlorophytum* under water tress observed that 5 day water stress caused an increase in activity of peroxidase in both leaf and tuber tissue but peroxidase activity in the tuber lowered while in the leaf trend increased continuously at severe water stress. Reedy *et al.,* (2004) in their work on water stress among five mulberry (*Morus alba* L.) cultivars observed that peroxidase activity along with catalase, glutathion reductase, SOD were significantly high in water stressed leaves and among five cultivars, S-13 and BC_2 –S9 had efficient antioxidative characteristics which provided better protection against oxidative stress under water stress. The increased activity of peroxidase was also reported in potato leaves under action of drought and polystimulin K (Nyzhnyk *et al.,*2004).

Adriano *et al.,*(2005) also reported that peroxidase activity increased in both leaves and roots of olive trees in severity of drought stress and concluded that the ability of olive trees to up- regulate the enzymatic antioxidant system might have been an important attribute linked to drought tolerance by cellular damage caused by ROS during water deficit. Increase in POD activity due water stress also reported by El-Tayab (2006) in *Vicia faba* cultivars, Klar *et al.,* (2006) in tissue of sweet pepper plants.

Altered lipid peroxidation by increasing peroxidase and superoxide dismutase activity due to water stress in *Vigna radiata* observed by Saleh *et al.* (,2007), Tetar and Gevrek, (2008) in Wheat. Dsouza and Devraj (2011) observed that enhanced drought caused increase in activity of POD indicating their role in detoxification of H_2O_2 in leaf and root tissue of *Dolichos lablab*. Also, isozymes pattern of POD in leaves indicated an enhanced expression of these genes rather than expression of newer ones. A new isozyme was observed in roots of stressed lablab. Similarly Patel and Hemantrajan (2012). observed that there was significant increase in POD activity in four genotypes of chickpea viz. Tyson, ICC 4958, JG 315 and ACP 923 and concluded that early drought stress (pre-anthesis drought) was more damaging than the late (Post anthesis drought).

Karimi *et al.,* (2012) observed that with increase in drought stress on caster bean cultivars, there was increased peroxidase activity at flowering stage than seedling stage. POD enzymes can efficiently remove H_2O_2 both in cytosol and chloroplast so

increase in the activity of this enzyme under drought stressed wheat lines perhaps shows the accumulation of H_2O_2 (Sharifi *et al.*,2012).

On the contrary, according to Zhang and Kirkham (1996) drought stress induces decreased cytosolic peroxidase activity in sunflower and *Sorghum* plants. Similar results also been reported in alfalfa by Iregoyen *et al.,* (1992) and in lettuce by Dina and Abdeal- Kader (2001).

In *Aristolochia,* we have recorded high values of phenolic compounds under stress. Thus, enhanced peroxidase activity may well be documented as an efficient H_2O_2 scavenging activity under stress and also it highlights the ROS under water deficit. The overall trend of increase in oxidases in this weed is in accordance with the findings of other workers also.

2. Catalase

The effect of water stress on activity of catalase in leaves of *A. bracteolata* Lam. is shown in Table 33 and depicted in figure 24. The activity of this enzyme is stimulated by drought stress in leaf issue of this plant. It is 1.45 mg H_2O_2 liberated $min^{-1}g^{-1}$ in control condition while an increase upto 2.04 mg H_2O_2 liberated $min^{-1}g^{-1}$ at severe water stress has been recorded.

Catalases eliminate H_2O_2 by breaking it down directly to water and oxygen. Plant catalases are haem enzymes that function as tetrameric proteins with 4 protophyrin moieties and exhibit a high molecular efficiency but a very low Km that ranges between 10 and 140 mM H_2O_2 (Feierabend, 2005). Catalases are localized in peroxisome where it functions chiefly to remove H_2O_2 formed, during photorespiration in C_3 species (Dat *et al.*,2000). but there are few reports of its localization in the mitochondrion and apoplast. Thus, they are very important to scavenge excess H_2O_2 (Pang *et al.*,2005).

Mittler *et al.,* (2002) stated that hydrogen peroxide concentrations generally increase in response to various abiotic stresses as takes part in the reactive oxygen regulatory network. Pandey *et al.,* (2013) concluded that in peroxisomes the CAT has an essential role in the removal of toxic H_2O_2 which is continuously formed during photorespiration by the dismutation of Superoxide radical converted in the NaDH

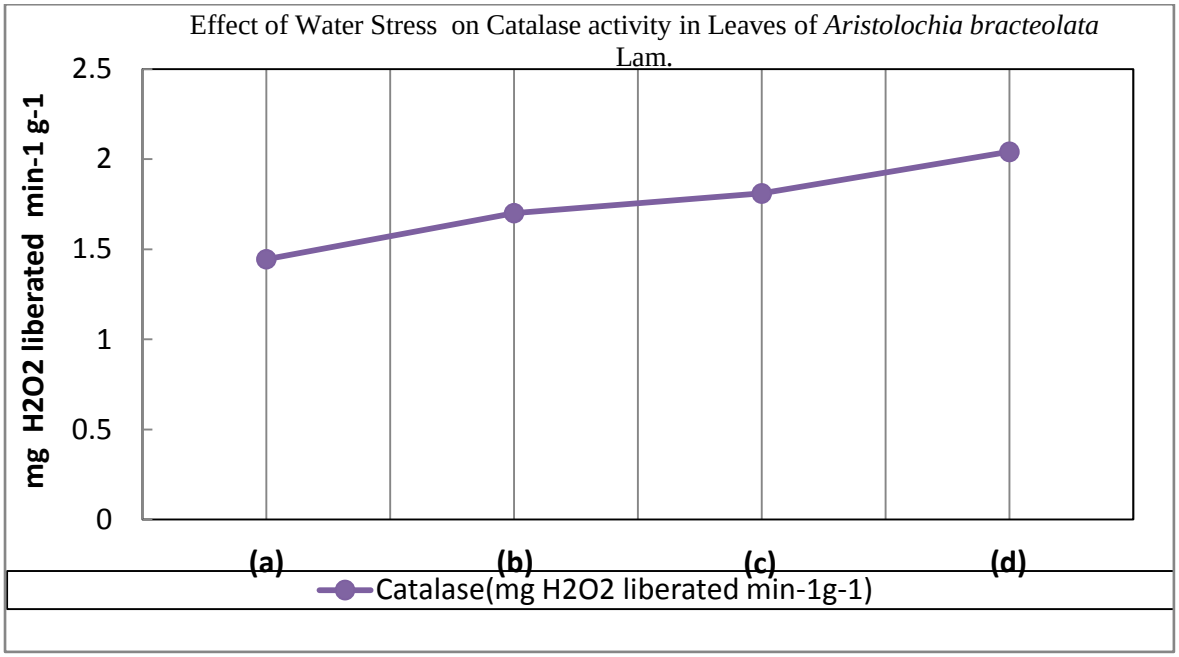
dependant ETS of the peroxisomal membrane by reducing senescence under moisture stress.

There are several reports showing an increase in catalse activity under water stress. Where Jagtap and Bhargava (1995) reported increase in catalase activity in drought tolerant variety of *Sorghum bicolor* when exposed to high light, low water, high temp. stress. Similarly, Sairam *et al.,*(1998) found that water stress cause an increase in catalase activity in wheat genotypes and concluded that H_2O_2 scavenging systems, as represented by ascorbate peroxidase and catalases, are more important in imparting tolerance against drought induced oxidative stress than SOD alone. The catalase activity in *Chlorophytum* is markedly increased in leaf and tuber tissue under moderate water stress conditions (Nikam, 2007) and concluded that an increase in catalase in leaf tissue of *Chlorophytum bulbosum* could be regarded as an indirect indicator of increase in photorespiratory rate under water stress. Water stress is found to cause an increase in catalase activity in leaves of wheat (Nayyar and Gupta, 2006) and in cowpea (Manivannan *et al.,* 2007). Similarly, Sadale (2007) on *Sesbania grandiflora* also observed stimulation of catalase activity in the young and mature leaves under drought conditions. Kirova *et al.,* (2004) noticed that two intensities of water stress (30 and 50%) caused an increase in the catalase activity in soyabean supplemented with nitrate and concluded that stress induced antioxidant enzyme activity was particularly dependant on source of nitrogen.

However, same cannot be said about the root or tuber tissue which does not carry out this process. Valentovic *et al.,*(2004) noticed increased catalase activity in the roots and mesocotyl, and concluded that these tissues were main sites of drought moderated H_2O_2 production. Also total catalase activity doubled under severe drought in two varieties of wheat is observed by Luna *et al.*(2005). According to them, CAT mRNA genes particularly at the level of translation allows control of leaf H_2O_2 accumulation at water stress.

Similarly, Abdalla (2011) observed significant increase in catalase activity in water stressed *Lupina albus* plants. Karimi *et al.,* (2012) observed similar increase in catalase activity in caster bean cultivars due to drought stress at flowering and in seedling stage. CAT is responsible for removal of excess H_2O_2 as it is averting the cellular damage under severe water stress conditions (Chang *et al.,*2012).

Figure 24 - Effect of Water Stress on Catalase activity in leaves of *Aristolochia bracteolata* Lam.



(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1days no water

(c) 48 hrs - Plants in pots with 42days no water

(d) 72 hrs -Plants in pots with 3days no water

An increase in CAT activity under water stress has been also reported by Huseynova *et al.,* (2010) in *Triticum aestivum*. Similarly, Bhardwaj and Yadav (2012) observed that there is increase in CAT activity in both drought tolerant and sensitive variety of horsegram. Patel and Hemantrajan (2012), observed significant increase in CAT activity in four genotypes of chickpea viz. Tyson, ICC 4958, JG315 and ACP 923 and concluded that early drought stress (pre-anthesis drought) caused more damage than the late drought stress (Post anthesis drought).

On the contrary, there are also reports showing significant decrease in catalase activity in several plants under study due to water stress. The pronounced decrease in catalase activity of drought stress (72-85 %) pea plants (Gogorcena *et al.*,1995). Castillo (1996), observed that catalase activity was sTable under mild water stress and was clearly inhibited under sever water stress in the inducible CAM plant *Sedum album*. Catalase activity decreased under water stress in Black pepper (Krisnamurthy *et al.,* 2000). According to Davis and Swanson (2001) catalase specific activity decreased in *Euphorbia esula* L. plants under 5 days water stress.

While Dsouza and Devraj (2011) reported steep decline in CAT activity of leaf and a moderate drop in roots of lablab in drought and suggested no significant role of this enzyme in stress.

In present findings a moderate rise in catalase activity has been recorded in *Aristolochia bracteolata* Lam. leaves under increasing levels of water deficit. As water stress has triggered secondary metabolism as a protective role in osmoregulation, the enhanced H_2O_2 levels might have resulted in increase in catalase activity thereby minimizing the scavenging effect of ROS. The increased levels of secondary metabolites indicate the reason for this catalase enhancement under stress to regulate the scavenging effect of ROS.

3. Indole Acetic Acid Oxidase

Effect of water stress on Indole Acetic Acid oxidase activity in leaves of *A. bracteolata* Lam. is depicted in Table 33 and figure 25. It is well evident from the results that increasing water stress shows positive relation with IAAO activity. When compared with control (0.215 Δ O.D. h^{-1} mg^{-1} protein) activity is increased three times (0.662 Δ O.D. h^{-1} mg^{-1} protein) under severe water stress.

Indole acetic acid oxidase, an enzyme which catalyzes the oxidation of indole acetic acid, is almost as widespread in nature as indole acetic acid and its levels of free IAA are, in turn, regulated via synthesis, binding, esterification, and enzymatic degradation catalyzed by IAA Oxidase (Schneider,1974). Different responses of certain oxidative enzyme have been observed depending on the different methods of water stress application on plant parts. The enhancement in activities of oxidative enzymes under water stress is possible, due to gradual shift of reductive metabolism to oxidative metabolism under these conditions.

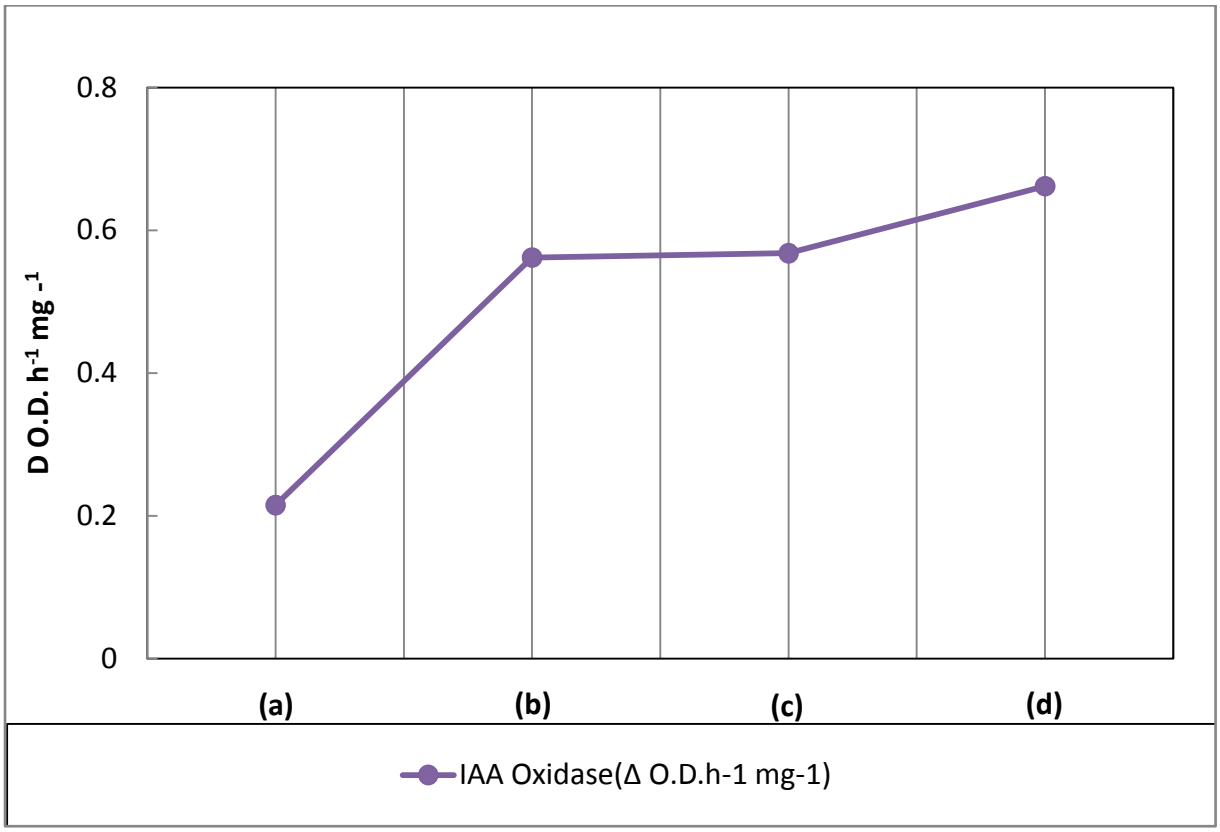
Goyal and Kochhar (1998) observed that IAA oxidase activities were inhibited by water stress in leaves of two genotypes of rice and Darbyshire (1971) had shown that the level of IAA oxidase activity may be affected by water stress in pea plants. These experiments showed that the reduced growth in stressed plants might be due to accelerated auxin degradation.

Sofo *et al.,* (2005) reported that the patterns of POD and IAA oxidase ran in parallel and showed increase in relation to the degree of drought in olive trees. The results indicated the ability of olive trees to regulate the enzymatic antioxidant system as it might be an important attribute linked to drought tolerance. Abdalla and El-Khoshiban, in (2007) observed that IAA oxidase enzyme activity increased progressively in the shoots and roots of weekly irrigated plants of both tolerant and sensitive cultivars by increasing the extent of stress so as to reach their maximum activities as being compared with those of untreated plant organs of *Triticum aestivum* and they concluded that increased activity of antioxidant enzymes due to stress was because of rapid removal of toxic oxygen radicals in tolerant cultivars than sensitive ones.

On the contrary, there are few reports for decline in Indole acetic acid oxidase activity. IAA oxidase systems in lam Porica and Kanking cultivars of winter wheat differed from each other in activity level but declined in response to water stress (Mills and Todd, 1973). According to Stutte and Todd, (1969) the maximum loss of IAA oxidase activity was of 50% when water deficit was nearing lethal point coincided with the behavior of POD in wheat leaves.

Thus, from above discussion and from obtained results, enhancement in activity of this antioxidant enzyme due to water stress in *Aristolochia bracteolata*

Figure 25: Effect of Water Stress on IAA oxidase activity in leaves of *Aristolochia bracteolata* Lam.



(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1 days no water

(c) 48 hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with3 days no water

Lam. is the response as activated defence mechanism against drought and to overcome drought by removal of toxic oxygen radicals.

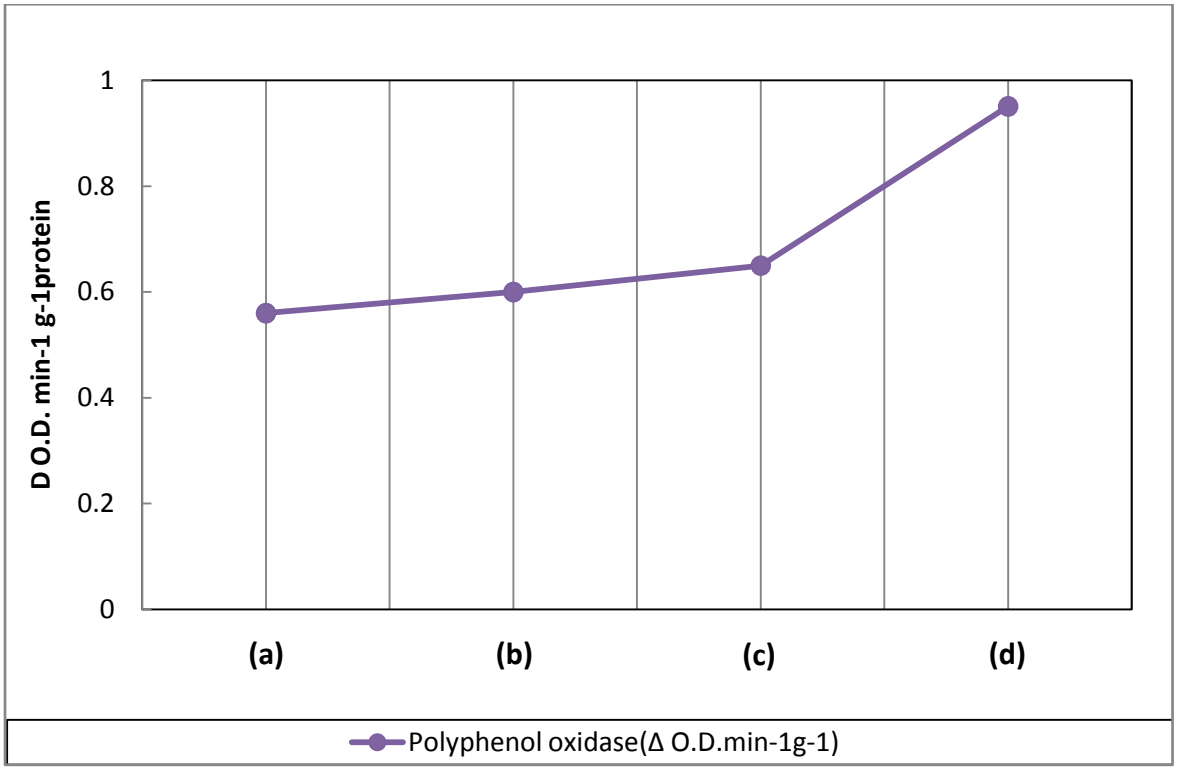
4. Polyphenol Oxidase

Effect of water stress on the activity of polyphenol oxidase in *Aristolochia bracteolate* Lam. leaves is shown in Table 33 and figure 26. It is clear from the data that activity of polyphenol oxidase has been with significant positive correlation with severity of stress. There is about 50% increase in polyphenol oxidase activity after 3 day water stress.

Polyphenol oxidase is a mixture of monophenol oxidase and catechol oxidase enzyme that is present in nearly all plant tissues. Also, it is a part of the plant anti oxidative system. Understanding the regulation and cell specific patterns of induced PPO expression sheds light on a possible role for PPO during plant water stress. PPO has been shown to be associated with drought and salt tolerance (Agarwal and Pandy, 2003).

Earlier, Bower and Lelyveld (1986) worked on the effect of long-term irrigation on avocado fruit polyphenol oxidase by appling soil moisture tension 80 kPa, 55 kPa and 35 kPa in the root zone. Soluble and total PPO activity decreased during softening of unstored fruits concluded that water stress affect postharvest polyphenol oxidase activity. Shivshankar (1988) showed that in coconut genotypes PPO activity induced during water stress. Chempakan *et al.,* (1993) reported that in coconut the activity of PPO was higher in susceptible varieties during stress. The induced leaf PPO activity alongwith increase in total phenol content during in water stress has been reported by Ashraf *et al*. (1994) in wheat leaves. Study of Banu and James (2008) on the effect of water stress on Polyphenol Oxidase activity in *Lablab purpureus* (L) suggested that enzymes like Peroxidase and Polyphenol Oxidase may serve as the indicator of water stress. Krishmurthy *et al.,* (2000) observed that Polyphenol Oxidase activity was higher due to water stress as there was upto 30% increase in Polyphenol Oxidase enzyme activity in black pepper. Similarly, Thipyapong *et al.,* (2004) reported that PPO suppression increased stress tolerance in tomato, also improved plant water relation, delayed photoinhibition and photoxidative damage during water stress. They further reported that among 7 member PPO gene family, PPO B and D are

Figure 26: Effect of Water Stress on polyphenol oxidase activity in leaves of *Aristolochia bracteolata* Lam.



(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1 days no water

(c) 48 hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with 3 days no water

transcriptionally and differentially activated in response to drought leading to increased PPO accumulation in leaves and stems of tomato.

Abdalla (2011) observed that antioxidant enzyme PPO was markedly stimulated in shoot and roots of two cultivars of *Triticum aestivum* as a response to water stress. While Dsouza and Devaraj (2011) in their study on water stress effect in root and leaves of *Dolichos Lablab* observed that drought enhances antioxidant enzyme PPO but more pronounced effect was noticed in root tissue and concluded that it helped in drought tolerance. These parameters and the recovery of plant against drought stress indicated substantial role of this enzyme towards drought response. Barbagallo *et al.,* (2012) observed that there is a dynamic balance between increased antioxidant pattern and Polyphenol Oxidase activity in tomato fruits under water stress. In this experiment Polyphenol Oxidase activity of tomato seemed to be related to the watering regime employed, showing a maximum rise in typical long storage cherry tomatoes under water stress.

Whereas opposite results to enzyme activation there are only few reports of inhibition of enzyme polyphenol oxidase as observed by Chakramurthy *et al.,* (2001) in tea leaves. Also, Nikam (2007) in *Chlorophytum bulbosum* plant noticed marked decline in the activity of PPO in water stress.

In present findings, the activity of polyphenol oxidase showed enhancement with increasing quantum of water deficit. As this enzyme acts as corrective measure against stress and associated with antioxidant role in scavenging ROS oxygen, its stimulation justifies the response of this plant towards water stress. Thus, drought resistant nature of *A. bracteolata* Lam. is clearly reflected by the behavior of increased PPO activity due to water stress alongwith increase in polyphenols specifically in leaves in this weed.

5. Nitrate Reductase:

Nitrate Reductase activity under the influence of water stress in *Aristolochia bracteolata* Lam. leaves is depicted in Table 34 and figure 27. Here in control NR activity is 0.15 nmole NO_3 formed h^{-1} mg^{-1}) whereas due to water stress it increased steeply up to seven times (0.76 nmole NO_3 formed h^{-1} mg^{-1}).

Nitrate being the most abundant form of inorganic nitrogen available for plants and since nitrate reductase is the first enzyme of the N- inorganic assimilation pathway makes this step fundamental in plant metabolism besides its activity being regulated by availability of water in soil (Meguro and Megulhaes, 1982). Nitrate taken up by plants is reduced to nitrite by NR.

NR represents the rate limiting step in nitrate assimilation and can be used an marker to estimate the capacity of plant roots and shoots to assimilate external N (Beevers and Hageman, 1969).

This enzyme catalyses reduction of nitrate to nitrite with pyridine nucleotide in N assimilation in higher plants (Ahmed and Ashdin, 1999). Nitrate reductase enzyme is highly sensitive towards oxidative stress and decreased activity might be due to breakdown of proteins under water stress conditions (Kalarani and Jayakumar, 1998).

Nitrate reductase (NR) is a multi-domain enzyme comprising the prosthetic groups molybdoprotein, Fe-heme, and FAD (flavin adenine dinucleotide) in a 1:1:1 stoichiometry that mediates an electron transfer from NAD(P)H to nitrate. The FAD and Mo-pterin domains function as the binding sites for NAD(P)H and NO_3^- respectively (Cannons,1993). NR was shown to catalyze a number of partial reactions including a dehydrogenase functionality typified by NADH-dependent reduction of ferricyanide and mammalian cytochrome C which could be inhibited at a sensitive thiol protected by NADH (Campbell, 1996).

The influence of water stress on NR activity has been studied in last few decades. To quote few, Nitrate Reductase activity decreased markedly with 20% water deficit in maize seedlings (Bardzik *et al.*,1971) and maize leaves (Shaner and Boyer, 1976). This observation is in agreement with Younis *et al.*, (1965) who found that maize seedlings grown under suboptimal water supply showed the same as even less nitrate level than control. Yadava *et al.* (1997) observed that reduced NR activity in leaf was reduced under water stress all the stages in both the cultivars and chickpea. Drought stress has negative effect on plant photosynthetic activity, N concentration and NRA is reported by many workers. Kathju et al., (1990) reported reduced N uptake from the soil and decreased activities of N assimilatory enzymes including nitrate reductase in plants growing in water stress. In Potato (Ghosh *et al.*, 2000),

Table 34 –Effect of water stress on Nitrate and Nitite reductase activities of *Aristolochia bracteolata* Lam. Leaves.

Enzyme	a	b	c	d
Nitrate reductase(nano mole NO_3 formed $h^{-1}mg^{-1)}$	0.15±0.02	0.15 ±0.02**	0.34 ±0.01*	0.76±0.02*
Nitrite reductase(nano mole NO_2 formed $h^{-1}mg^{-1}$)	0.33 ±0.11	0.30 ±0.10**	0.20±0.02**	0.13 ±0.01**

(a) Control – Plants in pots watered everyday.

(b) 24hrs –Plants in pots with 1 days no water

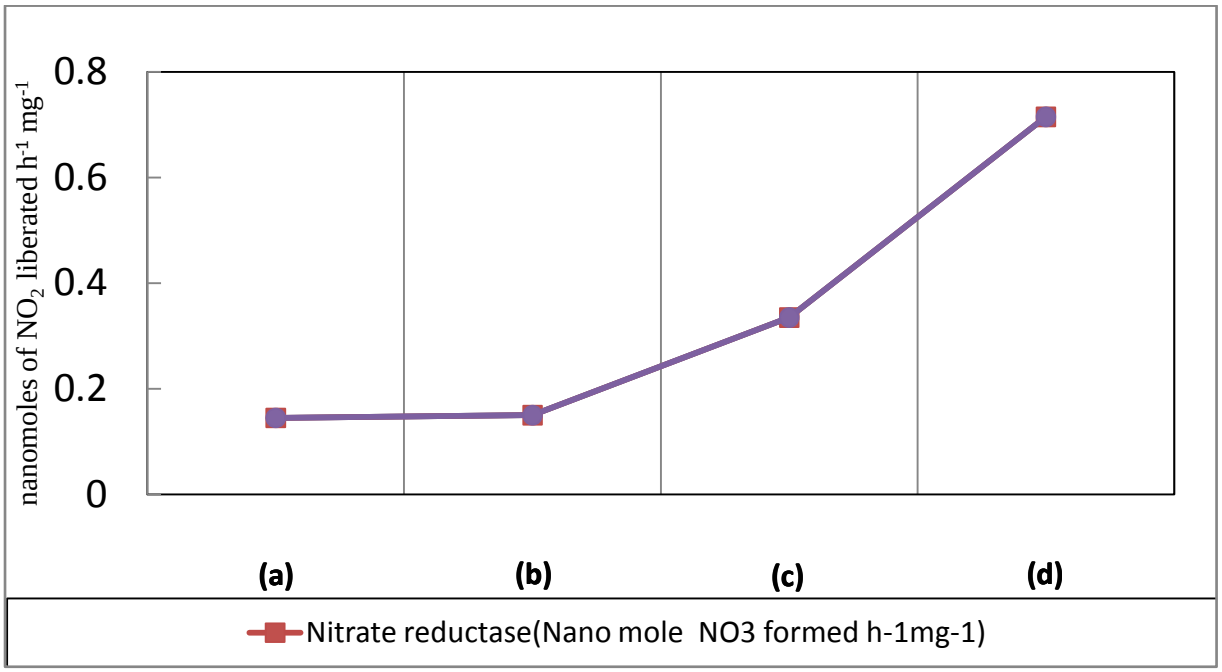
(c) 48hrs - Plants in pots with 2 days no water

(d) 72hrs -Plants in pots with 3 days no water

* - Values are significant at 5% level where $P < 0.05$.

** values are non- significant at 5 % level where $P < 0.05$

Figure 27 : Effect of Water Stress on Nitrate Reductase activity in leaves of *Aristolochia bracteolata* Lam.



(a) Control – Plants in pots watered everyday.

(b) 24hrs –Plants in pots with 1 days no water

(c) 48hrs - Plants in pots with 2 days no water

(d) 72hrs -Plants in pots with 3days no water

Nikam (2002) in tuber tissue of *Chlorophytum borivilianum*, in *Lymus chinensis* (Xu and Zhou, 2005). Xu and Yu (2006) also observed similar results in flag leaf and grains of winter wheat. Decreasing NRA of leaves under drought stress was observed when barley plants were exposed to the stress was observed by Krcek *et al.,* (2008).

The work of Diouf *et al.,* (2004) showed that water deficiency cause reduction in NR activity at all treatments in pearl millet and the effect was greater under high nitrogen supply. They concluded that as nitrate concentration under water deficit conditions had not been noticed increase in the reduction in NR activity was probably not due to limiting nitrates.

A decrease in the amount of total protein, decrease in their synthesis and a fall in nitrate reductase activity by the low nitrate influx under water stress was reported by Costa *et al.,* (2008) in his study of biochemical and physiological responses in Two *Vigna unguiculata* (L.) Walp. cultivars. Further, it was observed that there was decrease in leaf water potential, consequently lower transpiration levels and reduction in the influx of nitrate.

Rescently Pandey *et al.,* (2012) observed decreased NR activity in the leaves of *Avena* species (*A. Strigosa, A. bravis, A. vaviloviana, A. abyssinica, A.sativa, A. macrocana*, *A.sterilis*) in water stress and most pronounced effect was found in *A. sterilis*.

On the contrary, according to Arndt *et al.* (2001), high NR activity in the leaves of *Zizuphus rotundifolia* was paralleled by increase in proline concentration in severely water stressed plants. Also, Da-Silveira *et al.*(1999) observed a rapid increase in the nitrate content in the roots of cowpea plants due to water stress but showed a marked reduction in leaf NR activity and an increase in nodules. Similarly, Sadale (2007) observed that NR activity in all parts of *Sesbania grandiflora* increased under water stress.

In present study in *A bracteolala* Lam. an increase in NR activity is recorded in response to water stress. The overall N assimilation machinery is hampered under water stress and this is reflected in decrease in levels of total proteins. But nitrate, nitrogen and free amino acids showed marked rise. Thus one can predict that under stress protein N is being diverted towards synthesis of osmoprotectants like proline

and total ploypheno1s. Even reduced nitrite reductase (NiR) activity is in concurrence with present findings of other organic solutes.

6**. Nitrite Reductase (NiR) -**

The activity of Nitrite reductase (NiR) enzyme of leaves of *A. bracteolata* Lam. under the influence of water stress is shown in Table 34 and figure 28. It is observed that Leaf NiR activity inhibited due to water stress almost three times (0.13 nmole of NO_2 formed h^{-1} mg^{-1}) compared to control (0.330 nmole of NO_3 formed h^{-1} mg^{-1}).

Nitrite, the product of enzymatic reduction of nitrate, is further reduced to ammonia by the enzyme nitrite reductase. This enzyme is present in considerably higher quantities than nitrate reductase. From higher plants, the nitrite reduction was first demonstrated by Hageman *et al.,* (1962). The NiR enzyme protein is present in the chloroplast, probably in thylakoids, while in roots it is in the proplastids. It has a marked specificity for ferredoxin as an electron donor (Vega *et al.,*1980).

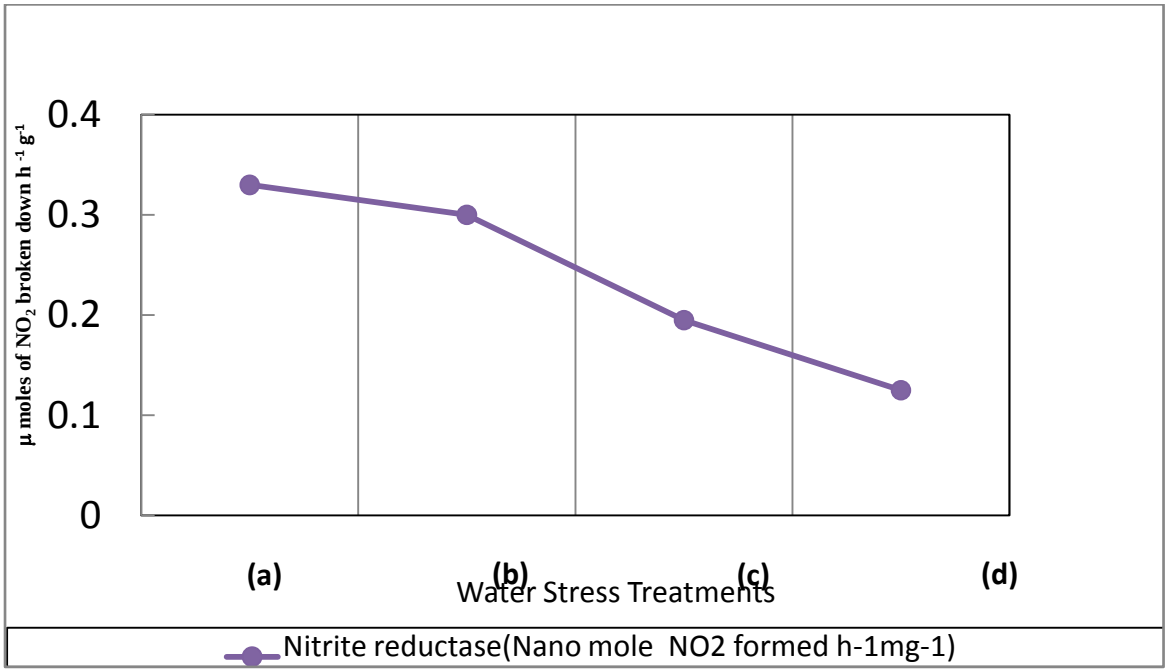
The six-electron reduction of sulfite to sulfide resembles that of the reduction of nitrite to ammonia. Both sulfite and nitrite reductases share an unusual prosthetic assembly in their active centers, namely siroheme covalently linked to an 4Fe-4S cluster (Crane and Getzoff, 1996).

Rosales *et al.,* in (2011) reported that nitrite is highly reactive, as plant cells immediately transport nitrite from cytosol into chloroplast in leaves and plastids in roots. In these organelles, nitrite is further reduced to NH_4^+ by NiR.

Very few reports are available which describe the behavior of NiR in plants under the influence of drought conditions. Patil (1995) observed that NiR activity decreased considerably in groundnut cultivars ICGS-11 and ICGS-44 under the water stress conditions. On the contrary, an increase in NiR activity in the roots as well as shoots of maize cultivars has been reported by Subramanian and Charest (1998) under the drought conditions.

Sadale (2007) observed an increase in NIR activity in young and mature leaves in *Sesbania grandiflora* while it decreased in roots due to water stress. It was concluded that probably the site of nitrite reduction changed to abiotic stress.

Figure 28: Effect of Water Stress on Nitrite Reductase activity in leaves of *Aristolochia bracteolata* Lam.



(a) Control – Plants in pots watered everyday.

(b) 24hrs –Plants in pots with 1 days no water

(c) 48hrs - Plants in pots with 2 days no water

(d) 72hrs -Plants in pots with 3 days no water

In leaves of *Aristolochia bracteolata* Lam. due to severe water stress activity of NiR enzyme has shown a perfect negative correlation. A decrease in NIR activity in leaves due to water stress might result into accumulation of nitrite as substrate may not be a limiting factor but some other cause may influence the decrease in NiR enzyme activity. It may be a consequence of equilibrium between the rates of synthesis and degradation, as the progressive tissue dehydration reduces the enzyme synthesis and activates enzyme inactivating system switching over the secondary metabolism and flow of NO_3^- diverted towards proline and alkaloids can be seen in our findings recorded elsewhere.

e) Photosynthesis

i) Photosynthetic pigments:

1) Chlorophylls:

The effect of water stress on *chl* a, *chl* b, total chlorophylls, chlorophyll a: b ratio and carotenoides contents in the leaves of *Aristolochia bracteolata* Lam. is shown in Table 35 and depicted in figure 29. It is evident that photosynthetic pigments decreased in the leaves of this plant under water stress conditions. It can be seen that with increasing level of water deficit in the soil medium, as well as in the tissue, there is remarkable decline in the level of green pigments. It is evident that chlorophyll a is largely affected by six days water stress. It decreases from 163.60 mg to 38.25 mg $100g^{-1}$ fresh tissue at 3 days of water stress. Chlorophyll b seems to be least affected by draught and it shows more tolerance. It can be seen that total chlorophylls which are 417.17 in the irrigated plants decreased to 209.24 in three day desiccated plants. Similar trend was observed in case of chl a/b ratio.

Chlorophyll and carotenoids pigments absorb radiant energy, which is used for photosynthesis. Chlorophyll is basically complex macromolecule with four porphyrin rings which contains magnesium and has an aliphatic alcohol phytol esterified to a propionic acid residue on one of the tetrapyrrole rings. Among the two chlorophylls present in higher plants Chl. a plays a primary role as reaction centre in photophosphorylation while Chl.b plays a secondary function. It is speculated that one of the 200 antenna Chl.a molecule associated with each PS – I reaction centre entrap a photon to initiate the process of photochemical charge separation (Golbeek, 1992).

Table 35 – Photosynthetic pigments in leaves of *Aristolochia bracteolata Lam.* Under Water Stress Conditions

Sr. No.	Parameter	Photosynthetic Pigments (mg $100g^{-1}$ fresh tissue)			
		a	b	c	d
1	Chl.a	163.60	156.01**	132.11*	38.25**
2	Chl.b	253.27	240.86**	214.22**	170.99**
3	Chl. a+b	417.17	396.87**	346.33**	209.24**
5	Chl.a/b	2.79	2.71**	1.85**	1.15**
6	Carotenoides	61.20	55.60**	49.20**	30.21**

(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1 days no water

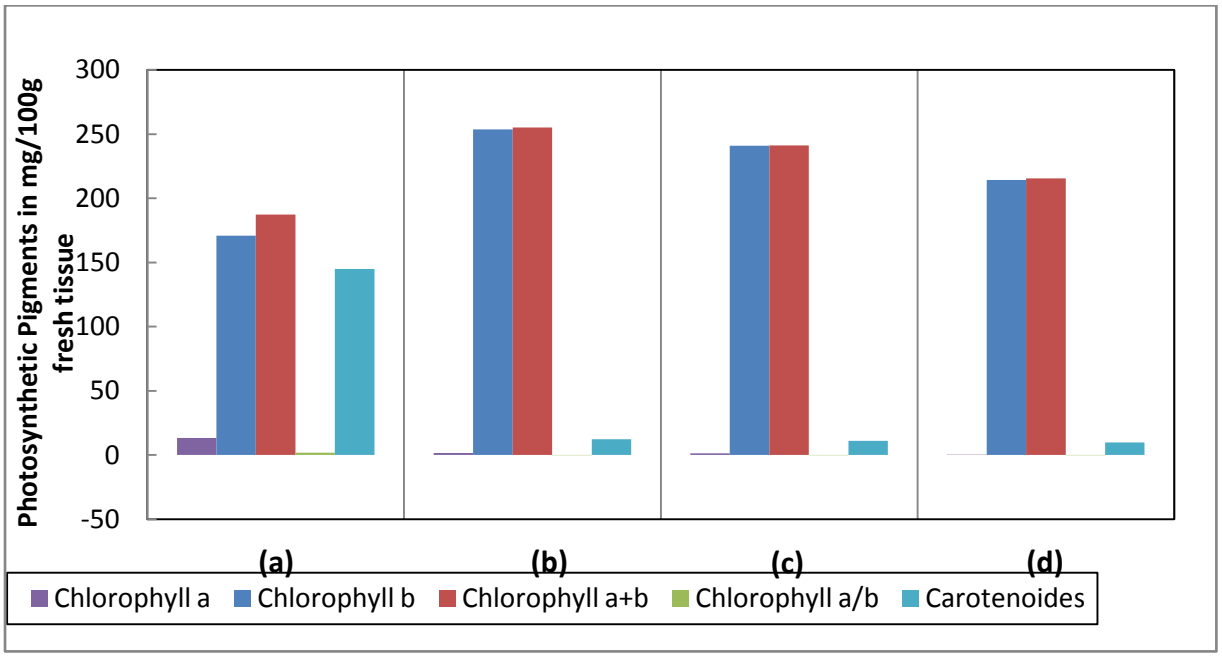
(c) 48 hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with 3 days no water

* - Values are significant at 5% level where $P < 0.05$.

** - Values are non- significant at 5% level where $P < 0.05$.

Figure 29: Photosynthetic pigments under water stress in leaves of *Aristolochia bracteolata* Lam



(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1 days no water

(c) 48 hrs - Plants in pots with 2days no water

(d) 72 hrs -Plants in pots with 3 days no water

The weight ratio of Chl a and Chl b is an indicator of the functional pigment equipment and light adaptation of the photosynthetic apparatus (Lichtenthaler *et al.*,1981) and decrease in Chl. a / b ratio by drought induction in cotton suggested that light harvesting complexes of thylakoid membranes was affected by short term drought (Parida *et al.*,2007).

Drought stress influenced on photosynthetic pigments and components (Anjum *et al.*, 2003), damaged photosynthetic apparatus (Fu and Huang, 2001) On the contrary, Kiani *et al.*(2008) postulated that drought stress decreased the tissue concentration of chlorophyllous cells.

The stability of chlorophyll molecules has long been considered as an essential parameter of stress resistance. The synthesis of chlorophylls can be highly sensitive to low leaf water potentials. Alberte *et al.,* (1977) found that loss of chlorophylls from maize leaves upon water stress amounted, failing to almost 60% of control, 8 days after irrigation. This loss of chlorophylls occurred mainly from mesophyll cells and could be accounted for by the losss of chloroplast membranes. A decrease in chlorophyll contents under water stress conditions has been recorded by number of workers. According to Goyal *et al.,* (2001) water stress affects the rate of photosynthesis, which in turn reduces the chlorophyll content in various genotypes of pearlmillet Whereas, Nyachiro *et al.,* (2001) reported that water deficit decreased chlorophyll a, b and total chlorophyll content in all cultivars of wheat and caused a decrease in photosynthetic efficiency.

Similarly, Chandrashekhar *et al.,* (2000) reported that chlorophyll content was reduced in hexaploid wheat varieties but increased in tetraploid wheat varieties in response to water stress. Jiang and Huang (2001) observed that RWC and chlorophyll content decreased with prolonged drought in two cool season turbgrasses with an increase in SOD, ascorbate peroxidase and glutathione reductase activities. The results suggest that drought manifected by decline in chlorophyll could be associated with decrease in antioxidant enzyme activities and increment in membrane lipid peroxidation. Water stress caused large reductions in leaf chlorophyll content in CV Ermela and small reductions in CV Consol (Colon and Yazzana, 2001).

The exposure of plants to water stress leads to a noticeable decrease in chlorophyll content is also observed in eggplants (Kirnank *et al.*, 2001), Black willow

(*Salix Nigra*) Li *et al.*,(2004) ,Soyabean (Inamullash and Isada, 2005), in *Sesbania grandiflora* Sadale (2007), in *Chlorophytum* (Nikam, 2002).

Similarly, Burman *et al*.(2004) observed that the water stress significantly reduced the total chlorophyll content in cluster beans at all stages of growth. Water stress significantly decreased leaf chlorophyll a, b as well as total chlorophyll and total chl pigments /cm^2 concentrations in *Matricaria chamolilla* L. (Pirzad *et al.*, 2011). Also, Khaleghi *et al.,* (2012) revealed that amount of leaf chlorophyll a and total chlorophyll was reduced by increasing water deficit in *Olea europaea* cv. Dezful. Shah *et al*. (2011) also observed decrease in chlorophyll a and b in leaves of cotton genotypes subjected to water stress. Similar results obtained by Changcheng *et al.*,(2011) in two shrubs, *Pyracantha fortuneana*, *Rosa cymosa*, and four trees, *Broussonetia papyrifera*, *Cinnamomum bodinieri*, *Platycarya longipes* and *Pteroceltis tatarinowii*. Drought stress significantly decreased pigments content, but increased the ratio of carotenoids to total chlorophylls in studied species. Moaveni (2011) studied drought stress in wheat leaves they observed decline in chlorophylls but differences are statistically not significant.

On the contrary, there are reports that chlorophyll content is increased significantly with water stress. Hamada (1996) observed that chlorophyll content increased in wheat subjected to drought and salinity. Sairam *et al.,* (1997) reported that tolerance of wheat genotypes to moisture stress is closely associated with its antioxidant enzyme system. They also observed that under water stress chlorophyll content is increased with antioxidant enzyme. Chlorophyll content increased with reduced availability of water as reported by Nativ *et al.,* (1999).They concluded that greater water use efficiency induced by drought could have resulted from stomatal regulation and increased chlorophyll content. Younis *et al.,* (2000) observed that short term water stress in the vegetative phase (7 days) improved the chlorophyll content in leaves of three sorghum cultivars while long term stress at vegetative and reproductive phases reduced chlorophyll content. Investigations of Ilakhun (2003) have shown that with soil drought, the chlorophyll content in plants increases, while photosynthetic activity gets lower in three cotton varieties.

Joshi (1985) had shown that chlorophyll concentrations in the leaves of *Poa pratensis* were not affected by water stress. Whereas, Thankmani and Ashokan (2002)

observed that total chlorophyll content was not affected however Chl. a / b content varied with water stress in six varieties of *Piper nigrum*.

From the forgoing discussion it appears that in most cases the chloroplast membrane system is destructed and degradation of chlorophylls and carotenoides takes place due to drought sensitivity.

It is well evident from the Table that in *Aristolochia* Chl. a has shown more severe degradation than chl. b under water deficiency and this has reflected in total chlorophyll content as well as chl. a/b ratio. Chlorophylls are primary biomolecules in light harvest in photosynthetic machinery. The effect of water deficit is not much significant on chl. b than chl. a. this indicates that photosynthetic light harvest machinery has not been damages severely but maintained its integrity thus, even the photosynthetic rate is lowered under water stress but still capable to continue it's photosynthesis. This control over damage may be attributed to carotenoid retention as well as osmoprotection of cellular membrane integrity through enhanced proline in water stressed plants.

2. Carotenoides :

The effect of water stress on carotenoid content in mature leaves of *Aristolochia bracteolata* Lam. is shown in Table 35 and figure 29. Decrease in carotenoid content is 4% in 6 day water stressed plants when compared to control. It is evident that still after stress, carotenoides reduced to half at severe water stress (61.20 mg $100g^{-1}$ fresh tissue) when compared to control (30.21 mg $100g^{-1}$ fresh tissue).

Carotenoids are a large class of isoprenoid molecules, which are *de novo* synthesized by all photosynthetic and many non-photosynthetic organisms (Andrew *et al*., 2008). They are divided into the hydrocarbon carotenes, such as lycopene and β-carotene or xanthophylls (Jaleel *et al.,* 2007). Out of the two carotenoid classes, carotenoids show multifarious roles in drought tolerance including light harvesting and protection from oxidative damage caused by drought. They are essential components of photosynthetic antenna and reaction centre complexes. Thus, they play a significant role in absorption, transfer of light energy for the process of photosynthesis, prevent direct quenching of triplet electron exited from reaction

centre, the generation of singlet oxygen and protects from oxidative damage of chlorophylls. Carotenoids participate in energy dissipation and can aid plant resistance against drought stress.

Decrease in carotenoides plays a significant role in hampering photosynthesis rate, thereby reduces the yield and the productivity of plants. Drought is a harmful phenomenon for plants which may induce irreversible damage or death in case of severe stress. Little attention has been paid to the drought effect on pigments. In their analysis of drought effects in bean plant (*Phaseolus vulgaris*), D'Arcy-Lameta *et al.,* (1996) revealed a decrease of both chlorophylls and of α- and β-carotenes together with xanthophylls..

On the contrary, there are also reports of accumulation carotenoides in stress conditions. A different pattern in the distribution and relative enrichment in the xanthophylls was due to the appearance of antheraxanthin and zeaxanthin as observed in drought-stressed *Nerium oleander* by Demmig *et al.,* (1988) and by Brugnoli and Bjorkman (1992) in *Gossypium hirsutum.* Wang *et al.,* (2001) reported that the carotenoid content in leaves of winter wheat increased under drought stress. In addition, carotenoid accumulation in chickpea plants under stress has a positive effect on the RWC is reported by Gunes *et al.,* in 2008. They suggested that high RWC may result from osmoregulation by osmoprotectants, as carotenoids or sugars are often accumulated in plants subjected to drought stress. Progressive increase in carotenoid content was found with the increase of drought increments in sunflower is reported by Nazarli *et al.* (2011).

Recently, Talebi (2013) observed that drought-tolerant chick-pea genotypes had higher relative water content, chlorophyll and carotenoid concentrations among 35 selected genotypes and the total carotenoid concentration was significantly lower in most chickpea genotypes.

Increased content of carotenoids is important for stress tolerance including light harvesting and protection from oxidative damage caused by drought observed by (Jaleel *et al.,* 2009). Oxidative damage generated by drought stress in the plant tissue is alleviated by a concerted action β- carotenes, ascorbate, α-tocopherol, reduced glutathione and enzymes including superoxide dismutase, peroxidase, ascorbate peroxidase, catalase, polyphenol oxidase and glutathione reductase (Prochazkova *et al.,* 2001)

From the forgoing discussion it appears that in most cases carotenoides are distructed which may be taken as a sign of drought sensitivity. On the other hand, *Aristolochia bracteolata* Lam. has a well developed capacity of carotenoid retention even under adverse drought condition. Simultaneously, reduction may be to protect cells from the cytotoxic effect of active oxygen radicals with non-enzymatic antioxidant system like carotenoides.

Thus, it is concluded that alongwith chlorophylls, carotenids content retention under drought supports drought resistant feature of *Aristolochia bracteolata* Lam.

f) Secondary metabolites under water stress:

1) Free Proline

The proline content in leaf, stem and root of A. *bracteolata* Lam. under the influence of water stress are presented in the Table 36 and depicted in figure 30. In *Aristolochia*, under normal control conditions highest proline content is recorded in leaf (1.45 mg / gm), followed by stem (0.79 mg / gm) and root (0.46 mg / gm). The water stress showed overall accumulation of proline with increase in water deficit. The stress showed significant rise in proline content in leaf followed by stem and root. With increasing water deficit (72 hrs) again there is overall accumulation of proline content in root from 0.46 to 3.31 mg / gm, in stem 0.79 to 4.10 mg / gm and in leaf from 1.45 to 4.76 mg / gm. It is well evident from the Table that even with water stress the general trend of proline accumulation has remained the same. That is root proline content has highest while the lowest one's are recorded in leaf. Proline concentration is increased 8 times in root at severe stress compared to control, 6 times in stem, and 3 times in leaf at higher water deficit.

Plants having a larger capacity to adjust unfavorable conditions show proline accumulation as this metabolite works as an osmotic adjuster thus consequently reducing the stress effects on plant metabolism (Nogueira *et al.*1998) and further this accumulation may be due to decrease in leaf water potential (Nanjo *et al.* 1999).

Proline a highly water soluble amino acid normally accumulates in higher plants in response to enviromental stresses (Turk & Demir, 2002). It is well known that free proline accumulates in plants in response to various types of environment stresses such as drought, salinity, high temperature, nutrient deficiency and exposure to heavy metal and high acidity (Oncel *et al.,* 2000).

Table 36 -Effect of water stress on free Proline content in different parts of *A. bracteolata* Lam

Plant Part	Free Proline (mg/ g dry tissue)			
	a	b	c	d
Leaf	1.45 ± 0.01	2.07 ± 0.04*	4.06 ± 0.02*	4.76 ± 0.01*
Stem	0.79 ± 0.01	1.45 ± 0.03*	3.31 ± 0.01*	4.10 ± 0.02*
Root	0.46 ± 0.02	0.91 ± 0.03*	2.40 ± 0.01*	3.31 ± 0.00*

(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1 days no water

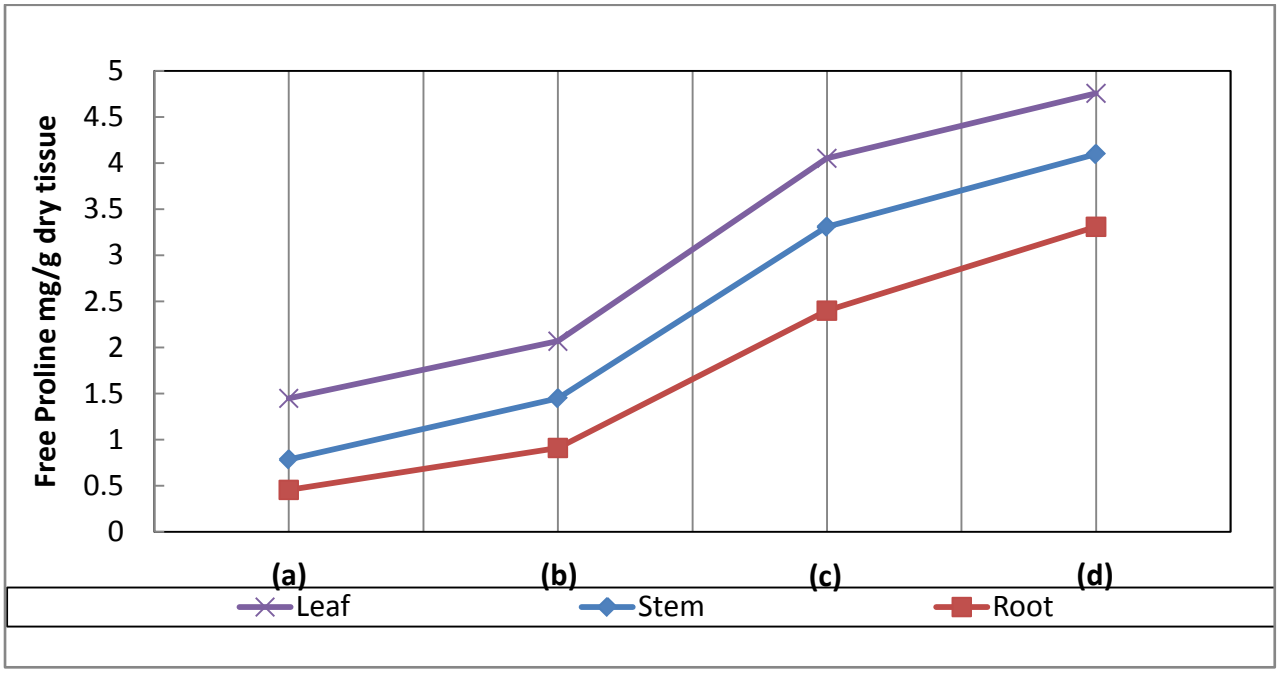
(c) 48 hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with 3 days no water

* - Values are significant at 5% level where $P < 0.05$.

* - Values are non- significant at 5% level where $P < 0.05$.

Figure 30: Effect of water stress on Free proline content in leaves of *Aristolochia bracteolata* Lam



(a) Control – Plants in pots watered everyday.

(b) 24 hrs –Plants in pots with 1 days no water

(c) 48 hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with 3days no water

It is the metabolite contributing mainly to osmoregulation, is accumulated mainly in the chloroplast and cytosol and cytoplasm. Vurayai *et al.,* (2011) suggested that although proline's role in plant osmotic tolerance remains controversial, it is however thought to contribute to osmotic adjustment, detoxification of ROS and protection of membrane injury during water stress. Beside this, in 2010, Shahraji *et al.,* postulated that proline might play a more complex role in conferring drought resistance than simply contributing to osmotic adjustment. It also involves in the regulation of cellular redox potential, reducing stress induced cellular acidification and priming oxidative respiration, associated with increased maintenance during water stress and to provide osmolyte balance needed for recovery (Ditmarova *et al.,* 2010). It has been shown that proline plays a key role in stabilizing cellular proteins of osmoticum of higher proline content in wheat plants in water stress (Khalil *et al.,* 2010).

Proline, which occurs widely in higher plants and accumulates in larger amounts than other amino acids (Abraham *et al.,* 2003) and regulates the accumulation of usable proline in cytosol where it contributse substantially to the cytoplasmic osmotic adjustment (Ketchum *et al.,*1991).

Special function proposed for proline is the stabilization of membranes under water deficit or a role as a small compatible solute (Delauney and Verma, 1993). Consumption of one ATP and two $NADPH^{+}$ per proline molecule implies that proline accumulation may serve as an electron sink mechanism.

Many workers have given ideas about accumulation of prline in respose to stress. Few of them described below. Proline is considered to play a major role in adjustment to osmotic stresses (Voctberg & Sharp, 1991).On the contrary; Thomas (1990) has argued that the principal role of proline may be probably not to reduce the osmotic potential, but to protect enzymes against dehydration and salt accumulation. In addition to sugar, proline accumulation acts as signaling molecule to activate multiple responses that are part of adaptation process (Clussen, 2005). According to Hong *et al.,* (2000) Proline accumulation may result from either induction of proline biosynthesis or inhibition of its oxidation because there is directally proportional relationship was observed between the proline content and the hydric deficit produced on the plants in many of experiments. Proline accumulates in cytosol and does not interfere with enzymatic reactions. Under water stress, the proline concentration can

reach up to 80% of the total amino acid pool in some plants. The synthesis of these compounds by the plant enhances tolerance to drought. (Zhang *et al.*,2004).

Under water stress plant synthesize osmoprotectants like ABA, amino acids, proline and prolamines, quaternary amino acids glycine betaine, mannitol, sorbitol, raffinose, H_2 O_2 , α–tocopherols etc. compound and it was discovered that accumulation of nitrogenous compounds under stress conditions and was found that many genes responsible for these processes (Hussein *et al.*,2011).

There are several reports on proline accumulation under water stress. The very first report regarding the free proline accumulation in rye grasses due to water stress was by Kemble and Mac-Pherson (1954).There was report that an increase in proline concentration in stressed plants might be primarily due stimulation of proline biosynthesis was proposed by Rhodes *et al.* (1986). Proline accumulation of in leaf tissues of *Sorghum* and sunflower plants under water stress observed by Jones *et al.*,in 1980. Similar results were found by Tusan *et al.* (2007) in his study on *Triticum aestivum* L., Ismail *et al.* (2004) in *Musa* species, Sadale (2007) in *Sesbania grandiflora* L. In another experiment *Vigna uguicalata* L. plants subjected to stress showed an increase in proline content as this solute is an osmotic adjuster as in the findings proline in the sempsevesde cultivar increased 45.32 % while in the Pituuba cultivar the increase was 57.27% (Costa *et al.,* 2008).

Also Ashraf & Iram (2005) noticed high accumulation of free proline in roots of *Phaseoles vulgaris* and *S. acceleato* under water stress conditions. Phutela *et al.*(2000) showed due to water stress proline content increased in leaves and roots of *Brassica juncea* cultivars differing in drought tolerance. But they proposed that the effect of water stress on the plant water relations and proline metabolism is genotype dependant.

Water stress results in stomatal closure and reduced transpiration rate, decrease in photosynthesis and water potential of plant tissue, growth inhibition and accumulation of abscisic acid (ABA), proline, mannitol, sorbitol and synthesis of new proteins and mRNA's (Lichtenthaler *et al.,* 1981). Jain *et al.* (2006) reported accumulation of proline in pigeon pea roots under water deficit.

Enhanced proline accumulation in plants in response to salt or water stress may have a role in tolerance mechanism at genetic level. According to Sharma and Verslues (2010) the levels of proline accumulation is in response to PEG-mediated low water potential are substantially higher than those typically seen in salt stressed seedlings of *Arabidopsis* mutants**.** It may be because of higher upregulation of *P5CDH* by low water potential**.** On the contrary, Nikam (2002) observed that in *Chlorophytum* water stress caused proline content decline in leaf tissue whereas no marked alterations in tuber.

In case of *A.bracteolata* Lam. also proline accumulation is evident in root tissue after short term water stress. With increasing water deficit free proline water content has shown marked accumulation in leaves and stem of this weed. This accumulation of proline acts a osmoprotectant maintaining membrane integrity as this is reflected in our findings of water relations (Table No.36). In primary investigations also similar proline accumulation has been reported in same plant by Murumkar, (1993) and this can be attributed as physiological adaptation towards water deficit. The fact that this weed thrives well in dry semi-arid region of this area, and dominates weed flora in summer season, proline probably contributes to drought tolerance process in this plant under unfavorable conditions.

2. Total Polyphenols

The total polyphenol content in leaf, stem and root of *A. bracteolata* Lam. under the influence of water stress is shown in the Table 37 and depicted in figure 31. In *Aristolochia,* highest Polyphenol content is recorded in leaf (4.18 mg/gm), followed by stem (2.09 mg/gm) and root (1.22 mg/gm). The water stress showed overall accumulation of total Polyphenols with increase in water deficit. The stress showed most significant rise in Polyphenol content in root followed by stem and leaf as with increasing water deficit (72 hrs) there is overall accumulation of Polyphenol content in root from (1.22 to 8.18), in stem (2.09 to 10.27) and in leaf (4.18 to 12.35 mg/gm). It is well evident from the Table that even with water stress the general trend of Polyphenol accumulation in different plant parts has remained the same. That is leaf polyphenol content has highest while the lowest is recorded in root. Thus, from the present findings it is evident that the polyphenol content has increased considerably in all parts but more pronounced effect is seen in root tissue when

exposed to water deficit. As in this weed, perennial roots are present and these are rich in Aristolochic acid this finding is more significant.

Phenols play a variety of roles in plants as they are the most plentiful class of secondary metabolites. They act as powerful antioxidants in plant tissue under stress (Sgherri *et al.*,2004).They form the pigments in flowers and fruits, contribute to biotic and abiotic stress tolerance, provide UV shelter and have role in pollen fertility (Martens and Mithofer, 2005). Leopoldini *et al.*(2004) stated that polyphenols are also acts as powerful reactive oxygen species scavengers alongwith caratenoides and proline. Polyphenolics compounds are also reported to cause stimulation of protein synthesis (Greppin and Horn, 1969) and promotion of ammonia elimination (Letan, 1967). Taiz and Zeigar, 2006 reported that Shikimic acid pathway participates in the biosynthesis of most of plant phenolics. Several phenolic compound in *Crataegus laevigata* and *C.monogyna* plant possess antioxidant activity and may help to protect cells against the oxidative damage caused by free radicles (Kirakosyan *et al.*, 2003).

Water stress increased the concentration of plant phenolics in drought-resistant (CHD 12, CHD 147) and drought sensitive (CHD 220, CHD 247) spring triticales (Hura *et al.*, 2009). They concluded that accumulation of phenolics may be participating in the mechanisms of adaptation of the photosynthetic apparatus to water defi cit in leaf tissues. Shah *et al.*, (2011) found that polyphenols were increased in all cotton genotype under drought condition (Parida *et al.*, 2007) observed that drought increased total free amino acids, proline, sugar as well as polyphenols significantly in moderately tolerant cotton genotype (GM 0903004) than the sensitive (Ca/H631) and concluded that polyphenols act as main compatible solutes in order to maintain osmotic balance, to protect cellular macromolecules, to detoxify the cells and to scavenge free radicals under water stressed conditions. Jadhav (1984) found that total polyphenols in flag and mature leaves of Proso millet increased to considerable extent when exposed to water deficit. Salunkhe (1990) reported a remarkable increase in polyphenol content of *Dodonea viscosa* leaves under water stressed condition. Similar results were observed in water stressed plants of *Thymes capitatus* observed by Delitala *et al.,* in 1986 and they further concluded that increased level of phenols in water stressed plant might play a role in regulations of transpiration and nutrient assimilation.

Table 37 - Effect of water stress on total Polyphenol content in different plant parts of *A. bracteolata* Lam.

Plant Part	Total Polyphenol content (mg/ g fresh tissue)			
	a	b	c	d
Leaf	4.18±0.02	5.57±0.02**	6.96±0.01**	12.35±0.03**
Stem	2.09±0.02	3.65±0.01**	5.22±0.03*	10.27±0.54*
Root	1.22±0.06	2.78±0.02*	4.00± 0.57*	8.18±0.02*

(a) Control – Plants in pots watered everyday.

(b) 24hrs –Plants in pots with 1 days no water

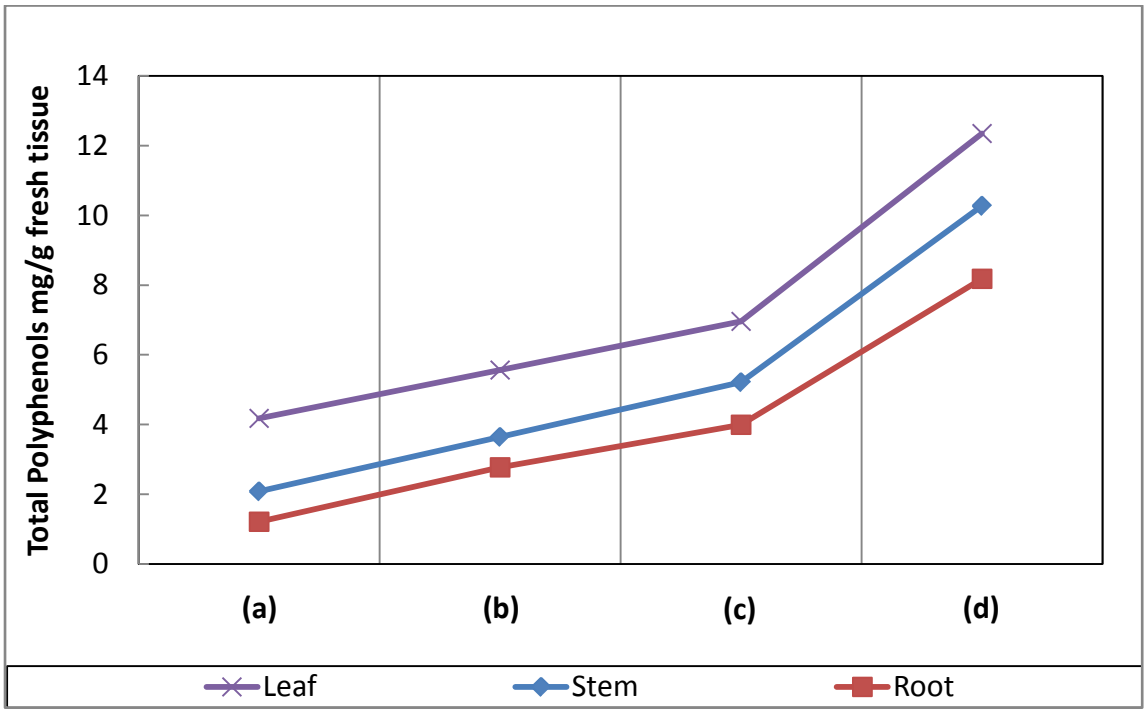
(c) 48hrs - Plants in pots with 2days no water

(d) 72 hrs -Plants in pots with 3 days no water

* - Values are significant at 5% level where $P < 0.05$.

* - Values are non-significant at 5% level where $P < 0.05$.

Figure 31: Effect of water stress on Polyphenols in *Aristolochia bracteolata* Lam.



(a) Control – Plants in pots watered everyday.

(b) 24hrs –Plants in pots with 1 days no water

(c) 48hrs - Plants in pots with 2 days no water

(d) 72 hrs -Plants in pots with 3 days no water

While Sadale (2007) evaluated that concentration of polyphenols in mature leaves of *Sesbania grandiflora* which showed a positive correlation with increasing water stress.

There are also reports of increase in polyphenol content like in Safflower seedling (Yaginuma *et al.,* 2003) as well as tea cultivars (Chakraborthy *et al.,* 2001). Similarly, Abreu and Mazzafera (2005) reported increase in level of phenolic compounds in medicinal herb *Hypericum brasilience* due to water stress.

Whereas Cheruiyot *et al.,* (2007) have concluded that polyphenols as potential indicators for drought tolerance in six tea clones. Recently Shah *et al.*,(2011) reported that polyphenols were increased under water stress where drought tolerant varieties of cotton have maximum amount of carotenoids along with polyphenols and vice versa for susceptible varieties.

On the contrary, ealier Tsai and Todd (1972) observed about 25% decline in phenolic content in both resistant and susceptible varieties of wheat due to water stress. According to them phenolic compounds were involved in celluler injury and suggested that it might be affecting various enzyme activities following drought stress. Ahmed *et al.* (1989) reported that drought condition reduced the total polyphenol content in the leaves of cotton while it had no effect on individual phenol compound in the roots. Ilieva and Radeva (1992) recorded reduction in ploy phenol content in cowpea plant under prolonged drought stress.

According to Patil (1995) polyphenol content in all genotypes of groundnut decreased due to water stress and the response of these plants also varied with respect to polyphenol accumulation under the soil moisture stress conditions. Similarly Sugar as well as total polyphenol content among the grapes was higher at fruit maturity in the stressed treatment (Peterlunger *et al.,* 2005).

Yildiz-Aktas *et al.* (2009) reported that water deficit the polyphenol compounds reduced in both cultivators of cotton. However, the reduction was less significant in the tolerant cultivar as compared with sensitive one.

However, the exact role of polyphenols during water stress is not completely understood. In present findings increment in total polyphenol content water stress in *Aristolochia bracteolta* Lam. is noticed in all parts namely root, stem and leaves. The effect is more pronounced in roots. These findings indicate that to control membrane

and cellular injury and ROS. The polyphenol content is on rise. This is also because of diverting nitrogen from other organic solutes. The enhanced activities of various oxidase like peroxidases, catalases and polyphenol oxidases also justify present findings.

3. Tannins:

The tannin content in leaf, stem and root of A. *bracteolata* Lam. under the influence of water stress are presented in the Table 38 and figure 32. In *Aristolochia* plant highest tannin content is recorded in leaf (21.35 mg/gm), followed by stem (16.29 mg/gm) and root (9.00 mg/gm). The water stress has resulted its accumulation. The water stress has resulted its overall accumulation. The stress showed significant rise in tannin content in leaf followed by stem and root. With increasing water deficit (72 hrs) again there is overall accumulation of tannin content in leaf from 21.35 to 31.46 mg/gm and in stem 16.29 to 26.41 mg/gm and in root from 8.989 to 21.910 mg/gm). It is well evident from the Table that even with water stress the general trend of tannin accumulation has remained the same. That is leaf tannin content has highest while the lowest ones are recorded in root. However, tannin accumulation is more profound in roots than leaves.

Tannins are group of plant secondary compounds which are known and used by man for centuries. The tannins are a heterogenous group of high molecular weight phenolic compounds with the capacity to form reversible and irreversible complexes with proteins, polysaccharides, alkaloids nucleic acids and minerals etc. (Schofield *et al.,* 2001).The tannins have been traditionally divided into two groups condensed and hydrolysable tannins. Hydrolysable tannins are made up of carbohydrate core whose hydroxyl group is esterifies with phenolic acids and condensed tannins (Muller - Harvey, 1999).

Plants containing tannins as one of its main phytochemicals are astringent in nature and are used to treat intestinal disorders (Dharmananda, 2003). The high tannin production may be an important strategy for *Casurina equosetifolia* in arid and infertile environment (Shao *et al.,* 2009).

Table 38 -Effect of water stress on Tannins in different plant parts of *A. bracteolata* Lam.

Plant Part	Tannins mg /g dry tissue			
	a	b	c	d
Leaf	21.34 ±0.02	23.59± 1.16*	25.84±0.21*	31.46±0.02*
Stem	16.29 ±0.02	19.10±0.03*	21.91±0.01*	26.40±0.03*
Root	8.98 ±0.02	11.79±0.02*	14.60±0.17*	21.91±0.11*

(a) Control – Plants in pots watered everyday.

(b) 24hrs –Plants in pots with 1 days no water

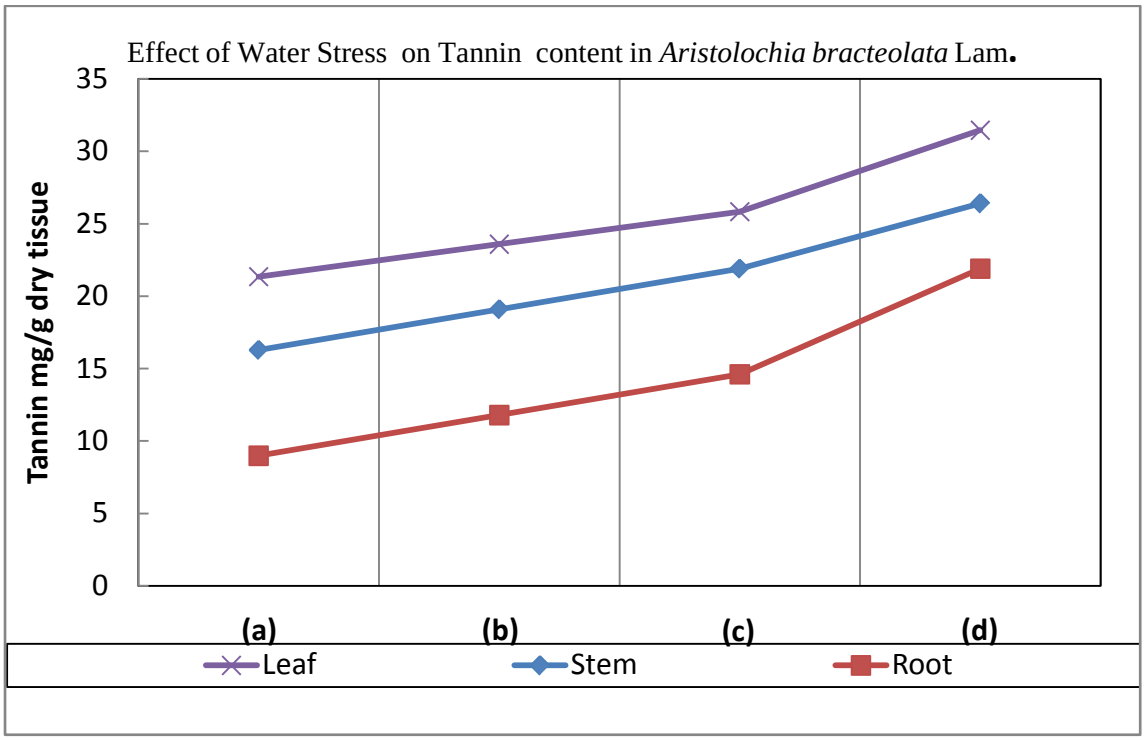
(c) 48hrs - Plants in pots with 2 days no water

(d) 72hrs -Plants in pots with 3 days no water

* - Values are significant at 5% level where $P < 0.05$.

* - Values are non-significant at 5% level where $P < 0.05$.

Figure 32: Effect of water stress on Tannin content in leaves of *Aristolochia bracteolata* Lam.



(a) Control – Plants in pots watered everyday.

(b) 24hrs –Plants in pots with 1 days no water

(c) 48hrs - Plants in pots with 2 days no water

(d) 72hrs -Plants in pots with 3 days no water

Increased tannin content in leaves of *Ceratonia siliqua* due to levels of stress reported by Kouki and Marieta (2002). El-Sayed *et al.* (2008) also reported that saponin content in *Trubulus* increased when subjected to water stress. Such an increase has been related to its protective role against oxidative stress (Lin *et al.* 2009). Tannin potential of the leaves of *Ocimum gratissimum* L and *Gongronema latifolium* Benth increased significantly as influenced by water stress (Osuagwu *et al.*,2010). Effects of fertilization and drought stress on tannin biosynthesis of *Casuarina equisetifolia* seedlings branchlets studied by Zhang *et al.(*2012).

The results showed that nitrogen and phosphorus fertilizer showed accumulation of total phenolics and extractable condensed tannins in branchlets of *Casuarina equisetifolia* Increased concentration of tannin in tissues of plants in response to the effects of water stress was also documented by other researchers. Hoshino and Ducan (1982) in

Sorghum plants; El- Shelk *et al*.(1999) in faba beans, Attia *et al*.(2007) in red wine grape. On the contrary results of the quantitative analysis in *A. wilkesiana* leaves showed that plants under drought tannins production was decreased (Odjegba1 and Alokolarol, 2013).

Thus positive correlation of enhancement in tannin content with respect to drought in plant *Aristolochia bracteolata* Lam. is indicative of protective oxidative role of tannins under drought. As many of the medicinal properties of this weed are known in tribal medicine this will definitely help to undertake research towards fortifying their medicinal value genetically as well as to enable them to tolerate high levels of drought and salinity which otherwise would affect the synthesis of phytochemicals like aristolochic acid and many others in this plant.

E) Antimicrobial Studies

i. Antibacterial Studies:

Antibacterial activity of root, stem and leaf extracts of *Aristolochia bracteolata* Lam. in aqueous, petroleum ether, methanol and butanol against pathogenic bacteria recorded in Table 39 and Plate 6. Different extracts of *Aristolochia bracteolata* Lam. screened showed growth inhibitory activity against bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Antibacterial activity was assayed by disc diffusion method and all extracts showed zone diameter of inhibition in the range of 7-18 mm around the disc.

Among four extracts methanol extract had maximum activity against most of the pathogens while aqueous extracts showed minimum activity.

Methanolic root extracts showed maximum inhibitory activity against *Escherichia coli* (18mm) followed by butanolic leaf extracts with zone of inhibition 14mm and methanolic stem extracts with zone of inhibition 12 mm.

While in case of *Pseudomonas aeruginosa* maximum inhibitory activity was observed in butanolic stem extracts with average zone diameter of inhibition upto 10 mm. All the root and leaf butanolic and aqueous extracts showed inhibition against *Pseudomonas aeruginosa* but aqueous stem extracts of *Aristolochia bracteolata* Lam. does not showed any zone of inhibition. Antibacterial activity against *S. aureus* by disc diffusion method showed maximum 13 mm inhibition zone diameter with methanolic root extracts, 13 mm with butanolic stem extracts and 9 mm with petroleum ether extracts. In stem extracts only butanolic extract showed 13mm zone of inhibition.

Different workers studied antimicrobial studies in medicinally important plants. Hinou *et al.* (1990) reported cytotoxic and antimicrobial principles from the roots of *Aristolochia longa*. The screening of secondary metabolites has shown that higher plants represent a potential source of new anti-infective agents (Kelmanson *et al.*,2001). Britto (2001) reported that most of antibacterial principles were either polar or non-polar and were extracted through the organic solvent medium. The present investigation suggested that the antibacterial principle can be extracted into organic solvent. Negi *et al.,* (2003) reported antibacterial activity of dried extracts of *Aristolochia bracteolata* Lam. against few gram positive and gram negative bacteria. All the crude extracts showed a broad spectrum of antibacterial activity. Kavitha and Nirmaladevi (2007) assessed Aristolochia *bracteolata* Lam. leaf extracts for its biotherapeutic potential. Both observed that among Aqueous, methanol and chloroform extracts, methanol extract was found to have the significant activity followed by chloroform extract against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas fluorescens* bacteria. Similarly Parveen and Rao (2012) in their work on antibacterial activity and phytochemical analysis of leaf

Table 39- Antibacterial activity different extract in Root, Stem and Leaf Extracts of *Aristolochia bracteolata* Lam.

Plant part	Solvent for Extraction	Test Organisms		
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
		ZOI (mm)	ZOI (mm)	ZOI (mm)
Root	Aqueous	-	9	11 ^{ns}
	Pet.Ether	-	9	8 ^{ns}
	Methanol	18*	7 ^{ns}	13 ^{ns}
	Butanol	12*	7 ^{ns}	11 ^{ns}
Stem	Aqueous	-	-	-
	Pet.Ether	-	7	-
	Methanol	12*	7 ^{ns}	-
	Butanol	10*	10 ^{ns}	13 ^{ns}
Leaf	Aqueous	-	7	-
	Pet.Ether	7	7	9 ^{ns}
	Methanol	9*	8 ^{ns}	-
	Butanol	14*	8 ^{ns}	8 ^{ns}
Positive Control	Ampicillin	45	11	20

ZOI- Zone of Inhibition

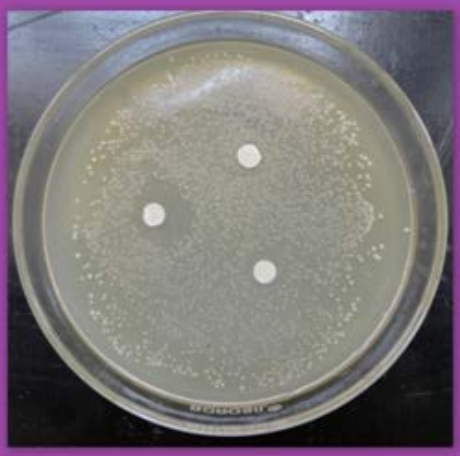
All the values of Zone Of Inhibition are mean of nine readings.

* - values are significant at 5% level. Where $P < 0.05$.

** - values are non-significant at 5% level. Where $P < 0.05$

Plate 6 - Antibacterial activity of different extracts of *Aristolochia bracteoltata* Lam.

A) Zone Of Inhibition Shown By *P. aeruginosa* in methanolic leaf extract of *Aristolochia bracteoltata* Lam.



B) Zone Of Inhibition Shown By *E.Coli* in butanolic Leaf extract of *Aristolochia bracteoltata* Lam.

C) Zone Of Inhibition Shown By *E.Coli* in methanolic root extract of *Aristolochia bracteoltata* Lam.

D) Zone Of Inhibition Shown By S. aureus in methanolic root extracts of *Aristolochia bracteoltata* Lam.

extracts of *Aristolochia bracteolata* Lam. revealed that aqueous extracts had significant inhibitory activity against *Staphyloccus aureus, Bacillus Subtilis, Escherichia coli, Klebsiella pneumoniae* bacteria followed by chloroform extracts along with phytochemical analysis of leaf extracts revealed relative distribution of the secondary metabolite responsible for potent antibacterial activity. Tesuchiya and his coworkers (1996) concluded that flavonoids are found to be effective due to their ability to form complex with extracellular and soluble proteins and with bacterial cell wall, more lipophilic, flavonoides may also disrupt microbial membrane.

Thus, in conclusion, leaf contains large amount of bioactive compounds as it showed maximum inhibition followed by root and stem extracts. Amongst all solvent methanol prove to be good solvent for extraction of antibacterial principle. When compared with previous reports our research also showed good antibacterial activity in different parts like root, stem and leaf of *Aristolochia bracteolata* Lam. Thus the present study supports that some medicinal plants show antibacterial activity and plant parts also contain important secondary metabolites. The presence of bioactive compounds might be a crucial factor for the antibacterial activity in *Aristolochia bracteolata* Lam. The occurrence of various secondary metabolites in medicinal plants may be containing antibacterial compounds.

Aristolochia bracteolata Lam. is traditional medicinal plant in India, and a rich source of bioactive compounds with diverse chemical structure. In the present investigation, different extracts of *Aristolochia bracteolata* Lam. showed good antimicrobial activity. It may be taken up for further isolation of pure bioactive compounds and identification and characterization for better management of microbial infection and multiple resistances in bacteria.

ii. Antifungal Studies:

Antifungal activity of the Aqueous, Petroleum Ether, Butanol, and Methanol extracts of root, stem, leaves of *Aristolochia bracteolata* Lam. against test fungi *Aspergillus wentii, Fusarium monoliformae, Penicillum imblicatum* is presented in Table 40 and Plate 7 represents. It is well evident from the Table that extracts of *Aristolochia bracteolata* Lam. screened showed growth inhibitory activity against test fungi. Different extracts showed moderate antifungal activity with the average zone of inhibition by 7-15 mm by disc diffusion method.

Methanolic leaf extracts showed maximum zone of inhibition against *Aspergillus wentii* (15 mm) but petroleum ether extracts does not showed any zone of inhibition. Butanolic and aqueous leaf extracts were also effective against *Aspergillus wentii*. In case of stem extracts only methanol showed 8 mm Zone of inhibition against *Aspergillus wentii* and in root extracts Butanol was effective with again 8 mm ZOI and petroleum ether with 7 mm ZOI. Whereas no zone of inhibition were found in aqueous and methanol root extracts.

Fusarium monoliformae was not susceptible for any root extract. It showed only ZOI in butanolic stem and methanolic leaf extracts (8 mm ZOI) which are lower as compared to positive.

While *Penicillum imblicatum* showed maximum ZOI (12 mm) in aqueous leaf extracts and minimum 7 mm in methanolic leaf extracts. Thus leaf extracts were effective against *Penicillum imblicatum* followed by root and stem extracts of *Aristolochia bracteolata* Lam. In stem extracts only methanolic extract is effective with 9 mm ZOI. In root extracts all extracts except butanol showed inhibition zone with 8 mm, 8 mm, and 9 mm in aqueous, petroleum ether and methanol extracts, respectively.

Thus from all extracts leaf extract was found to be more effective against test fungi may be due to supposed more amount of bioactive compound present in it followed by root extracts of *Aristolochia bracteolata* Lam. Whereas only butanolic stem extracts is effective against *Fusarium monoliformae* and methanolic stem extracts showed inhibitory action against *Aspergillus whentii* and *Penicillum imblicatum.*The screening of secondary metabolites has shown that higher plants represent a potential source of new anti-infective agents (Kelmanson *et al.,* 2001). According to the study of antifungal activity assessment of leaf of *Aristolochia bracteata* Lam. studied by Kavita and Nirmaladevi (2007), different extracts (Aqueous, methanol and chloroform) were effective against fungal strains like *Aspergillus Niger, Aspergillus terreus, Penicillium notatum* and *Rhizopus stolonifer* and concluded that the tested fungal strains were more susceptible to aqueous extract followed by methanol extract and chloroform extract. Ramsubramania *et al*. (2011) studied phytochemical, pharmacognostical and antifungal activity of *Aristolochia bracteolata* Lam. and concluded that bioactive principles present in the extracts may

Table 40- Antifungal activity using different extracts in Root, Stem and Leaf Extracts of *Aristolochia bracteolata* Lam.

Plant Part	Solvent for Extraction	*Aspergillus wentii*	*Fusarium monoliformae*	*Penicillum imblicatum*
		ZOI (mm)	ZOI (mm)	ZOI (mm)
Root	Aqueous	0 ± 0.00	0 ± 0.00	8* ±0.57
	Pet.Ether	7±0.00	0 ± 0.00	8 ±0.57
	Butanol	8* ±0.57	0 ± 0.00	0 ± 0.00
	Methanol	0 ± 0.00	0 ± 0.00	9* ±0.47
Stem	Aqueous	0 ± 0.00	0 ± 0.00	0 ± 0.00
	Pet.Ether	0 ±0.00	0 ± 0.00	0 ± 0.00
	Butanol	0 ± 0.00	8* ± 0.00	0 ± 0.00
	Methanol	8 ±0.00	0 ± 0.00	9** ±0.57
Leaf	Aqueous	9 ±0.57	0 ± 0.00	12** ±0.47
	Pet.Ether	0 ± 0.00	0 ± 0.00	11 ±0.57
	Butanol	11** ±2.30	0 ± 0.00	8*±0.57
	Methanol	15 ±2.64	8* ± 0.57	7 *±1.15
Antibiotic	Nystatin	20 ±1.52	21±1.15	11 ±1.52

ZOI - Zone Of Inhibition (mm).

* - Values are significant at 5% level where $P < 0.05$.

** - Values are non- significant at 5% level where $P < 0.05$.

Plate 7 - Antifungal activity of Different extracts of *Aristolochia bracteoltata* Lam.

A) Zone of Inhibition shown by *Penicillum imblicatum* in aqueous leaf extracts of *Aristolochia bracteoltata* Lam.

B) Zone of Inhibition shown by *Fusarium monoliformae* in methanolic leaf extracts of *Aristolochia bracteoltata* Lam.

C) Zone of Inhibition shown by *Fusarium monoliformae* in butanolic stem extracts of *Aristolochia bracteoltata* Lam.

D) Zone of Inhibition shown by *Aspergillus whentii* in methanolic leaf extracts extracts of *Aristolochia bracteoltata* Lam.

be used in the treatment of ringworm infection. They also observed that ethanolic extract was effective against Trichiophyton *rubrum* and *Microsporum canis.*

It is well evident from present findings that *Aristolochia bracteolata* Lam., known as wormkiller also shows antifungal potency and this may be because of presence of Aristolochic acid. The Aristolochic acid a strong alkaloid is present in root, stem and leaf in lesser degree of purification. Present mechanism of inhibitory action of bioactive principles of *Aristolochia bracteolata* Lam. may be due to inhibition of fungal cell wall, protein and amino acids, sphignolipid biosynthesis and electron transport chain. The presence of bioactive compounds in this study might be a factor for the antibacterial activity of *Aristolochia bracteolata Lam.*

In the present investigation different extracts of *Aristolochia bracteolata* Lam. showed positive antifungal activity. There may be bioactive compounds from plant parts useful for better management of fungal infection and resistance towards pathogenic fungi Isolation and purification such compound may be useful for plant based drug preparatio

CHAPTER V

SUMMARY AND CONCLUSIONS

All weed species, which are part of dynamic ecosystems, originate in natural environment and become hurdle either to the crops or man. They show some diagnostic features such as large number of seeds, short seed dormancy period, high rate of seed germination, rapid seedling growth, high reproductive ability, short span life cycle, high environmental plasticity, self-compatibility, effective and efficient methods of dispersal of propagules, production of different types of novel allelochemicals and tolerance to biotic and abiotic stresses which enable them to grow and survive in varied habitats and inhospitable ecological conditions. As a result of this, these weeds become dominant throughout the world.

Weeds are adapted to habitats disturbed by man. They may be useful in some respects and harmful in others. They may be useful to some people and hated and despised by others. There are weed races of most of our field crops and these interact genetically with cultivated as well as truly wild races. This interaction probably results ultimately in better crops and more persistent weeds. Although, some weeds have evolved elegant adaptations under the influence of human activity, many wild species had weedy tendencies before man existed. Weeds are products of organic evolution. They are also genetically labile and phenotypically plastic. Several weeds grow abundantly, gregariously in the open spaces, roadsides, crop fields etc. and also grow in all type weather conditions. But rainy and winter seasons are most productive for their growth and spread. By careful screening and analysis, these weeds would be of great source of herbal medicines. The role of weeds in the present pharmacopeia has been overlooked, despite significant evidence that weeds in particular, are an important source of medicines for indigenous people and have a highly significant over representation in indigenous pharmacopoeias in relation to other types of plants.

On the other hand, weeds are the biggest competitor for economic crops, lawn, grass and open places left for beautification. They cause tremendous losses to production. The struggle for existence between weeds and man has been going on from time immortal. It must be kept in mind that nature favors weed species which have large ecological amplitude. Such species multiply and flourish even in adverse environmental conditions such as edaphic factors, microclimate and change the succession of weed

species as well. The ecological amplitude of a plant species may get reflected in its morphological, anatomical and physiological behavior which is flexible over time.

Baramati is a drought prone semi-arid region as it is severally affected by rain shadow effect of Western Ghats. *Aristolochia bracteolata* Lam**.** is one of the common dominant weed of this region. The occurrence of this weed in a variety of habitats and soil types reveals its uniqueness which has prompted us to understand this weed in more elaborative way. Keeping this in mind, we felt it worthwhile to study the eco-physiology of *Aristolochia bracteolata* Lam. as this plant is growing well even under unfavorable conditions like extreme hot summer of this region. Hence, it was also essential to probe into various physiological processes and microbial studies of this weed.

In present study, *Aristolochia bracteolata* Lam. plant is studied to know its life cycle pattern, habitat, vegetative characters and yield output. In present investigation stress is given to study of some biochemical processes like mineral nutrition, photosynthesis, nitrogen metabolism enzymology etc. Work is further extended to study ecophysiology of this plant to understand it's success of survival in arid conditions and this involves study of germination behavior, growth pattern, water status, the mode of photosynthesis, metabolism of organic solutes as well as role of secondary metabolites like free proline, total polyphenols and tannins etc. With these physiological and biochemical parameters, the probable cause of its success as a dominant weed is being found out.

As this weed known for medicinal value also, study is further extended to focus more light on production and utilization potential of some novel components of medicinal importance because now a days there is an increasing interest worldwide in herbal medicines. Such studies encompass investigations of physiological parameters, analysis, pharmacological properties of bioactive compounds and their ability to treat various diseases. In present study, *Aristolochia bracteolata* Lam. is also screened for its antibacterial and antifungal potency as well as some important secondary metabolites or biomolecules responsible for this as only few reports are available in this regard, still so far.

Aristolochia bracteolata Lam. grows luxuriantly even in summer season and this clearly sheds light on it's hardy nature. To understand the capability, in this weed water stress treatment was given to this weed and analysis of different parameters with respect to water stress was also carried out. The significant findings obtained during research and some broad conclusions from the results of the present investigation are summarized below –

a. Habitat analysis-

1. In present case, the success of establishment of this weed *Aristolochia bracteolata* Lam. lies in successful seasonal germination pattern, perennial root stalk, physiological capacity to drought tolerance, adapted life form and efficient dispersal of their fruits and seeds.
2. Rhizosphere soil analysis clearly showed that it prefers alkaline soil, low EC values indicate less free ion availability and poor soil condition, high availability of organic carbon favours for plant growth.
3. High $CaCO_3$ in rhizosphere soil of *Aristolochia bracteolata* Lam. further reflects on their influence of availability of free cations and anions in the soil thereby limiting soil fertility.
4. In present study, open field showed higher nitrogen content which may be useful for more N uptake than cultivated field which is further available for weed *Aristolochia bracteolata* Lam. where higher nitrogen content is observed in leaf tissues.
5. Low phosphorus content is found in open and cultivated soil. This fact may be supplemented with low microbial life in these soils.
6. Presence of higher content in open field sheds light on availability of high K from clay minerals than cultivated soil.
7. Amount of Ca is found highest than other minerals in different soils studied and this may be due to alkaline nature of soils. It helps in stabilizing the soil structure by replacing the adsorbed sodium and prevents damages to soil structure in arid region during water scarcity.

8. Magnesium content is at second high level in soils associated with *Aristolochia bracteolata* Lam. and justifies alkaline nature of soils.
9. Sulphur content is relatively less as compared to calcium and magnesium because it may be in bound form with Ca and Mg salts increasing soil sodicity.
10. In both the fields sodium is greater three times than that of potassium content confirms sodic saline condition leading to potassium depreciation.
11. In soils studied, iron content and its availability is low due to high pH levels which decreases Fe^{3+} activity.
12. Zinc content is very low in the studied soils Copper content in both soils is within range of optimum levels recorded for normal soils. And so, these elements are not a limiting factor normal plant growth of this weed.
13. Soils were tested for biological activity through study of rhizosphere. It was revealed that Soils were rich in fungi like *Trichoderma viridae, Penicillium notatum, Aspergillus fumigatus, Mucor indicus, Rhizopus stolonifer, Humicola fuscorata, Fusarium* sp., *Stachybotrys* sp., *Cladosporium* sp., and *Acrimonium* Sp. from rhizosphere soil of open field. While in cultivated fields *Penicillium notatum*, *Aspergillus fumigatus* and *Alternaria alternata* alongwith *Cladosporium sphaerospermum, Humicola fuscorata, Acrimonium* sp. *Gleomastix* sp. were recorded. From the present study it may be concluded that, the arid land of Baramati region rich with fungal flora belonging to order Pleosporales and dark septate fungi like *Alternaria* and *Fusarium* spp. which are adapted to high temperature and high salt conditions.
14. Growth analysis showed that it is with underground fleshy erect stem with several perennial branches.
15. Seasonal variations in leaf water Status in *A. bracteolata* Lam. showed that high moisture content, mesophyll succulence index and surface expansion in summer than monsoon and winter season. High moisture content is because of high mucilage in leaf mesophyll tissue and moisture

content is maintained by development of leaf succulence index in this plant. All these findings indicate clearly CAM nature of this plant.

16. Seeds show dormancy, when different seed dormancy techniques were applied to study it's effect on germination, mechanical scarification showed 90% germination where as hot water treatment and stratification treatment showed only 40% and 60 % germination, respectively. It shows that in *Aristolochia* hard seed coat causes dormancy due to total impermeability of testa to water. This dormancy can be removed by mechanical scarification as higher germination is observed in mechanical scarification treatment.
17. Mineral nutrition was investigated in this weed. Overall inorganic elements like Nitrogen, Calcium, Sulphur, accumulated more in leaf while Phosphorus, Magnesium, Manganese in seeds whereas Potassium, Sodium, Iron, and Copper were found more in stem tissue. High zinc content was found in roots.
18. Nitrogen is accumulated more in shoot part, as translocation to shoot as more N content is recorded in leaf followed by stem. The accumulation of novel alkaloid Aristolochic acid in leaves and stem also justifies N accumulation in shoot.
19. Highest P content is recorded in leaf followed by stem i.e. in shoot part in *Aristolochia.* This gives clear idea that this weed is receiving adequate quantity of P from soil and thereby maintaining its normal metabolic activity.
20. Accumulation of more potassium in stem tissue of *A. bracteolata* Lam. helps in transport of solutes, maintains osmotic potential and helps leaves for osmotic adjustment.
21. Accumulation of Ca in leaf tissue of *Aristolochia bracteolata* Lam. is observed. It may take part in regulation of water flow across plant membranes thereby helps in stomatal movement to reduce water loss.
22. Magnesium content is more in stem as well as leaves and is minimum in roots. High Mg content in shoot part helps in K translocation. It helps for

synthesis of other nitrogen containing compounds like aristolochic acid which is chief constituent of this plant.

23. In *Aristolochia,* sulphur content is higher in leaves as well as stem and minimum in roots. The bitterness attributed to this weed may be also the effect of high sulphur content.
24. The pattern of accumulation of Sodium in different parts of *A. bracteolata* Lam. is highest in stem (717.45 mg/gm) > leaf (456.56 mg/gm) > root (239.15 mg/gm). Its high values in leaf indicate the adaptive mechanism acquired by this weed to survive in arid region by maintaining water integrity through succulence.
25. Fe content is recorded more in leaf and stem tissue of *A. bracteolata* Lam. as iron is important element in oxido-reduction reactions in plants it's performance justifies normal healthy growth of this weed in even dry arid regions.
26. In *Aristolochia,* Mn content is adequate in leaves to run normal photosynthesis even in arid condition during summer period. The high Mn content in leaves is indicative of active PEPcase activity in cytosol as a part of CAM behavior reported in this weed.
27. Zn content in *Aristolochia* is within the range of normal and this reflects normal metabolic activity of various oxidases.
28. The pattern of accumulation of copper in different parts of *A.bracteolata* Lam. is very low and as stem > leaf > in root. The role of Cu may play an important role in antimicrobial activity.
29. Organic constituents like Amino acids, total and soluble proteins, carbohydrates were studied in this weed. The pattern of accumulation of these organic constituents is in order of leaf > stem > root.
30. Leaves of *Aristolochia* have more amino acids thus reflects this as a major site of metabolic activity. The dominance of this weed in hot summer and it's survival may be because of synthesis of free amino acids like proline against membrane impairment as osmoprotectant and enhanced proteolytic activity.

31. As *Aristolochia* is a dominant weed, metabolic activities are occurring in leaf tissue and so reflecting high soluble protein content. The present findings clearly reveal that even in adverse environment, this weed thrives well because of it's undisturbed competitive metabolic strategy towards survival.

32. In present study, accumulation of reducing sugars can be attributed towards its role as osmoticum against osmotic adjustment on dry period. The high values of total carbohydrates in leaves are indicative of reduced transport mechanism towards the roots as these have recorded lower values. Enzymatic analysis of oxidases like POD, CAT, IAAO, PPO showed highest activity in leaves than root and stem.

33. High levels of peroxidase enzyme in *Aristolochia bracteolata* Lam.clearly indicate its active role in this weed and this can be attributed to effective defence mechanism in drought prone conditions.

34. Higher Catalase activity has been reported in leaves of *Aristolochia bracteolata* Lam. in present findings. As catalase is a predominant enzyme is scavenging ROS and acting as strong antioxidant, its high activity in hot arid xeric condition is quite justifying. Also, its occurrence in roots and stem indicates the general oxidative role as oxidation of H_2O_2 generated during catalytic activity of other metabolites.

35. High IAA oxidase activity in *Aristolochia* leaves along with peroxidase suggests that enhanced protective defence mechanism under arid conditions.

36. In present studies, higher activity of PPOs is observed in leaves than root and stem of *A. bracteolata* Lam. It may be towards plant defense mechanism against pests and diseases, regulating phenolic compounds and alkaloids.

37. In present findings the nitrate reductase activity is considerably high in leaves of *A. bracteolata* Lam. This indicates efficient nitrate metabolism and nitrogen turnover even in unfavorable conditions like hot summer. The role of this enzyme to supply N towards synthesis of aristolochic acid

and other N containing secondary metabolites will be worth to investigate further.

38. Nitrite reductase activity is higher in leaves followed by stem and roots in this weed. As NiR activity is many folds higher than NR, it reflects the efficiency of reduction of NO_2^- to NH_4^- thereby regulating N metabolism. This *Aristolochia* weed belonging to Aristolochiaceae is enriched by aristolochic acid, a secondary metabolite rich in Nitrogen and the efficient N metabolism a high NR, NiR activity may be responsible for directing N towards synthesis of this secondary metabolite.

39. Seasonal photosynthetic pigment composition in *Aristolochia bracteolata* Lam. leaves showed that *chl b* values are greater than *chl a* in all three seasons. In summer *chl. a* level has declined almost threefold than monsoon thereby altering *chl.* a / *chl.* b ratio. This shift is also indicative of change in the mode of photosynthesis i.e. C_3 to CAM as well as sensitivity towards photooxidation. While total chlorophylls, carotenoides content is greater in monsoon than winter and summer season. It seems from photosynthesis studies that the composition of photosynthetic pigments alongwith carotenoides in this weed is much more satisfactory to achieve maximum photosynthetic efficiency and ultimately results in maximum output in the form of high seed output as well as successful survival and establishment even under unfavorable conditions.

40. High succulence, moisture content values in leaves of this weed falls within the range of CAM plants.

41. Highest value of Chlorophyll Stability Index and Carotenoid Stability Index was recorded in monsoon while lowest in summer thereby suggesting a higher drought resistance during summer. However, low carotenoid stability index follows the same pattern and it also indicates the protective role of photooxidation of chlorophyll in adverse conditions like drought in summer.

42. Seasonal pattern of diurnal fluctuations in titrable acidity number and pH values were studied in the leaves of *Aristolochia bracteolata* Lam. A

diurnal fluctuation in TAN and pH was recorded in this weed and the same trend consisted in seasonal studies also. Studies on the stomatal behavior had been made and it showed that these remained open during night while closed during day time. All these findings strongly advocated that facultative CAM mode of photosynthesis prevails in this weed because this effect is more profound and significant in summer season than favorable rainy and winter period.

43. Hill reaction in isolated chloroplasts showed strong progressive reduction of DCPIP after 10 minutes and about 13 % reduction of DCPIP has been observed after 20 minutes. From the reduction percentage in Hill reaction in *Aristolochia bracteolata* Lam. leaves, it is clear that the slow rate of photolysis can be attributed to rate limiting photosynthesis.

44. The effect of photo-oxidation on photosynthetic pigment has been studied. This effect is more profound on *chl* b than *chl* a, with overall drastic decline in these two pigments. Light as well as heat has triggered overall oxidation of these photosynthetic pigments (total chlorophylls) and this effect is more severe in photo-oxidation than dark treatments. Photo-oxidation of chlorophyll pigments stimulated photoblenching in detached pigments whereas detioration of these pigments under heat and dark is much slower.

45. Among various secondary metabolites studied, leaf showed three times high proline content in shoot than root indicating it’s active role and action. Accumulation of proline in different parts may be towards osmotic adjustment of this plant to arid environment and more amounts in leaf tissue probably justifies its role as an osmoticum towards cell protection maintaining hydration of macromolecules by proline association.

46. Polyphenol content was also greater in leaves. It may be acting as defense mechanism against herbivores. Due to its bitter taste and herbaceous nature when this weed is exposed to open grazing field the weed may get protected and thus, avoided by herbivores.

47. Similarly tannin accumulation in leaf is almost three times higher than roots. From this, it is quite clear that occurrence of more amount of tannin in this weed may play its primary role towards defense mechanism and this may be further associated with medicinal properties of this plant.

b. Isolation and Characterization of Aristolochic acid

48. The separation and quantification of aristolochic acid by HPLC revealed that quantitative response of aristolochic acid in methanolic extract was more prominent as compared to ethyl acetate extract of root stem and leaf of *A. bracteolata* Lam. There is single fluorescent yellow spot in day light on TLC plate.

49. In mass spectrum of isolated aristolochic acid, observed molecular ion base peak was at m / z 295 amu and other molecular ion peaks were at m / z 311, 280, 265, 237, 209, 179, 163. From the mass spectra observed molecular wt. is 341.20 which are comparable with theoretical molecular wt. 341.27.

50. The infrared (IR) spectrum of isolated aristolochic acid showed prominent bands for the C - N stretching at 1630.04 cm^{-1}. N = O stretch signals at 1543.61 cm^{-1} symmetric stretch 1518.94 and 1595.44 cm^{-1} asymmetric stretch, (-OH) stretch showed strong broad signal at 2800-3700 cm^{-1}); Carbonyl group (C = O) having sharp signal at 1680.92 cm^{-1}, C-O stretching at 1262.84 cm^{-1} and C-H stretch at 748.32 cm^{-1}.

51. ^{1}H-NMR spectrum of isolated aristolochic acid showed signals at δ 7.258 and δ 7.928 attributed to H_7 and H_5 respectively. Two singlets at δ 6.433 and δ 8.664 were assigned to C2 and C9 protons. Presence of methoxyl group at δ 4.248 and methylenedioxy group at δ 5.334.

52. Isolated aristolochic acid purity observed as about 75.00 % by high pressure liquid chromatography (HPLC).

53. X-ray crystallographic analysis of isolated aristolochic acid crystals belong to monoclinic, space group P21/C, *a* (A^0) = 17.9200, *b* (A^0) = 5.2540, *c* (A^0) = 9.5700 (A^0), α = 90.0000(A^0), β = 106.2700(A^0), γ = 90.000 (A^0).

54. All characterization parameters showed that isolated aristolochic acid is A. acid I (8-methoxy-6-nitrophenanthro [3, 4-*d*][1, 3] dioxole-5-carbosylic acid) and molecular formula $C_{17}H_{11}NO_7$ with molecular weight 341.27.

c) Effect of water stress –

As *Aristolochia bracteolata* Lam. survives even under hot dry summer conditions, this weed was subjected to analyze the effect of water stress on various physiological and biochemical parameters. From the findings, drought resistant nature of *Aristolochia bracteolata* Lam. is well evident.

55. With increasing water stress, in *Aristolochia bracteolata* Lam. moisture content, succulence index, mesophyll succulence index showed a very meager decline in status, thereby showing its ability to maintain water status even under water deficiency. This may be one of the reasons for its success to survive under arid, dry and hot environmental conditions. But, at the same time water stress has caused a significant decline in surface expansion values in leaves and these values are in accordance with those reported for CAM plants. This feature points clearly towards CAM nature of this weed under drought.

56. Mineral adjustment of selective uptake, transport and redistribution may be a part of survival mechanism against water deficit. The leaf, stem and roots of *Aristolochia bracteolata* Lam. subjected to water stress showed accumulation of N, P, K and S in all parts of this weed. Whereas Mg and Ca declined in roots considerably. Iron, Zinc, Manganese and Copper accumulation more pronounced in roots.

57. Short term water stress is showing slight decline in N content. At severe water stress, there is accumulation of nitrogen. This may be due to

accumulation of N in free amino acids like proline as well as secondary metabolites like alkaloids and aristolochic acid.

58. Phosphorus content in leaf, stem and root of *Aristolochia bracteolata* Lam. has shown increase with prolonged water stress. The enhanced values of P in shoot that is leaf and stem reflect its lower mobility, accumulation of P compounds and enhanced proteolysis. This might be adaptive feature to overcome drought.

59. In present findings, with increasing water stress there is increase in K content, in *Aristolochia bracteolata* Lam. The enhancement is significant in shoot part especially in stem. Accumulation of potassium in shoot part is accounted for osmotic adjustment and this may be helping the regulation of metabolism and transport under unfavourable conditions.

60. In *Aristolochia bracteolata* Lam. also similar decline in Ca content in leaf and stem tissue and its accumulation in roots is recorded. This mobilization may be helpful to overcome drought stress.

61. Mg content has shown increment in both leaf and stem and slight decrease in root tissue. Retention of chlorophylls in the leaves under drought stress in this plant also supports these findings. Role of Mg as a cofactor for various enzymes related with energy transfer is well known. The high Mg content in water stressed *Aristolochia* points towards plant's adaptive response against stress.

62. With increasing water stress there is increase in the level of Sulphur in leaf and stem of *Aristolochia bracteolata* Lam. The increment may be attributed towards protective role of sulphur under water stress.

63. With increasing water stress there is overall decline in Na content and this effect is more profound in leaves as well as roots. Lowering of Na content coincides with increased K content under stress thereby enhancing the capability of the plant to sustain under water deficit and able to maintain higher content Na in leaf tissue but not in stem and root tissue.

64. Overall decline in Mn content in shoot part i.e. leaf and stem, while an increment is seen in root under increasing water stress in *Aristolochia bracteolata* Lam. Present findings clearly indicated mobilization of Mn from shoot part towards roots.

65. It is observed that with increasing water stress Zn has been mobilized from leaf and stem i.e. shoot part and accumulated in roots of *Aristolochia bracteolata* Lam. As Zn is regulating water uptake mechanism it may be repairing the membrane damage caused in roots under water deficit. This can be treated as an adaptive feature, also playing as a protecting ROS activity.

66. Due to water stress there is increment in Cu content in roots while it declined in shoot part i.e. leaf and stem. As Cu is involved in ROS activity through various oxidases, in present activity in roots and this may be against osmoregulation. It seems that due to water deficit Cu is mobilized from shoot part to roots and this may be a strategy of drought tolerance through osmoprotection.

67. Water stressed plants of *Aristolochia bracteolata* Lam. showed increase in activity of oxidative enzymes like Catalase, Peroxidase, Polyphenol oxidase, IAA oxidase. This indicated the accumulation of Reactive Oxygen Species and protection against water stress indicating activated drought tolerance mechanism in this weed.

68. Enzymes of nitrogen metabolism like nitrate reductase (NR) showed enhanced levels while nitrite reductase (NIR) activity is declined in leaves

of *Aristolochia bracteolata* Lam. with severity of water stress. The overall N assimilation machinery is hampered under water stress and this is reflected in decrease in levels of total proteins. But nitrate, nitrogen and free amino acids showed marked rise. Thus due to stress protein N is being diverted towards synthesis of osmoprotectants like proline and total polyphenols. Even reduced nitrite reductase activity is in concurrence with present findings of other organic solutes.

69. Photosynthetic pigments decreased in the leaves of this plant under water stress conditions. With increasing level of water deficit in the soil medium, as well as in the tissue, there is remarkable decline in the level of green pigments. The effect of water deficit is not much significant on chl b than chl a. and this indicates that photosynthetic light harvest machinery has not been damaged severely but maintained its integrity. Thus, even the photosynthetic rate is lowered under water stress it is still capable to continue it's photosynthesis. This control over damage may be attributed to carotenoid retention as well as osmoprotection of cellular membrane integrity through enhanced proline in water stressed plants.

70. Enhanced accumulation of secondary metabolites like proline, polyphenols, tannins was observed in highest water stressed plants. Proline accumulation was eight times in roots than in stem and leaf at severe water stress. *Aristolochia bracteolata* Lam. Accumulation of proline acts an osmoprotectant maintaining membrane integrity during water stress.

71. Overall accumulation of total Polyphenols was recorded in this weed with increase in water deficit. It showed most significant rise in Polyphenol content in roots followed by stem and leaf. This is may because of diverting nitrogen from other organic solutes. The enhanced activities of various oxidase like peroxidases, catalases and polyphenol oxidases also justify present findings.

72. The water stress has resulted overall accumulation of tannins of *Aristolochia bracteolata* Lam. The stress showed significant rise in tannin

content in leaf followed by stem and root. Enhancement in tannin content with respect to drought in this plant is indicative of protective oxidative role of tannins under drought.

d) Antimicrobial activity:

Fresh extracts in different solvent from leaf, stem and root extracts of *Aristolochia bracteolata* Lam. were tested for antimicrobial activity using test organisms.

Antibacterial activity

73. Root, stem and leaf extracts of *Aristolochia bracteolata* Lam. when screened for microbial activity showed growth inhibitory activity against bacteria like *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Amongst four extracts, of solvent from aqueous, petroleum ether, methanol and butanol against pathogenic bacteria, methanol extract had maximum activity against most of the pathogens while aqueous extracts showed minimum activity. Methanolic root extracts showed maximum inhibitory activity against *Escherichia coli* and *Staphylococcus aureus.* While in case of *Pseudomonas aeruginosa* maximum inhibitory activity was observed in butanolic stem extracts.

Antifungal activity

74. *Aristolochia bracteolata* Lam. showed growth inhibitory activity against test fungi. Different solvent extracts showed moderate antifungal activity with the average zone of inhibition by disc diffusion method. Methanolic leaf extracts showed maximum zone of inhibition against *Aspergillus wentii. Fusarium monoliformae* was not susceptible for any root extract. It showed only ZOI in butanolic stem and methanolic leaf extracts. While *Penicillum imblicatum* showed maximum ZOI in aqueous leaf extracts.

The above findings are indicating strategy of survival potential like high succulence, photosynthetic parameters like chlorophylls, carotenoides alongwith facultative CAM nature of this weed *Aristolochia bracteolata* Lam. Secondary metabolites are also useful for its survival and may also have role of medicinal importance biomolecule like aristolochic acid. Antimicrobial properties prove its medicinal potential. Thus, further investigations are necessary for isolation of bioactive compounds useful for better management of antimicrobial activity from this plant. Isolation and purification such compounds may be worth for herbal drug preparation.

The present study thus clearly indicates that this weed carries a great potential. The findings have showed that there are many more avenues of application of this important weed worth investigating. This dominant weed *Aristolochia bracteolata* Lam. will be a major source of research in drug chemistry in coming days.

CHAPTER VI

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PUBLICATIONS

List of Publications:

1. **Madhuri B. Kadam**., Sashikant. J. Chavan and Chandrashekhar. V. Murumkar, Report of facultative CAM in *Aristolochia bracteata* Retz. *Ad. Plant. Sci*. 25(II): 755-757,2012.

2. **Madhuri B. Kadam**., Subhash S. Deokule., Sashikant. J. Chavan and Chandrashekhar.V. Murumkar, Antimicrobial activity reported in a weed *Aristolochia bracteata* Retz, *J. Nat. Prod. Plant Resour*. 2012, 2 (5):589-592.

3. **Madhuri B. Kadam** and Chandrashekhar.V. Murumkar, Antifungal activity reported in Weed *Aristolochia bracteata* Retz.in Proceeding of State Level Seminar Organized by MPASC, College, Panvel on 1st Septebmer 2012.