"STUDIES ON THE ANTIMICROBIAL PROPERTIES OF THALLOID LIVERWORTS FROM WESTERN GHATS OF MAHARASHTRA."

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By

JAYRAM KESHAV KASHID

M. Sc., B. Ed

Under the Guidance of

DR. SHASHIKANT J. CHAVAN

M. Sc., Ph. D.

Associate Professor,

Post-Graduate Research Centre,

Department of Botany,

Tuljaram Chaturchand College, Baramati.

Dist. Pune - 413 102

Co-Guidance of

PROF. SUBHASH S. DEOKULE

M. Sc., Ph. D.

Professor and Head,

Department of Botany, University of Pune,

Pune - 411 007

Maharashtra, (India)

May 2012

CERTIFICATE

This is to certify that the work incorporated in thesis entitled **"Studies on the antimicrobial properties of thalloid liverworts from Western Ghats of Maharashtra"** submitted by Mr. Jayram Keshav Kashid, was carried out by the candidate under my supervision in the Post-Graduate Research Centre, Department of Botany, Tuljaram Chaturchand College, Baramati- 413 102 Dist. Pune, for the degree of Doctor of Philosophy. Such material obtained from other sources has been duly acknowledged in the thesis.

Place : Baramati.
Date : 31/5/2012

Prof. Subhash S. Deokule
Research Co-Guide

Dr. S. S. Deokule
Professor & Head
Department of Botany
University of Pune,
PUNE-411 007.

Dr. Shashikant J. Chavan,
Research Guide

Forwarded through –

Tuljaram Chaturchand College Baramati

TULJARAM CHATURCHAND COLLEGE
BARAMATI-413 102 (PUNE)

DECLARATION

I hereby declare that the work incorporated in the thesis entitled **"Studies on the antimicrobial properties of thalloid liverworts from Western Ghats of Maharashtra"** has not been submitted in part or full by me for any degree or diploma of any other University or institute.

Jayram K. Kashid,
Research Student

Place : Baramati.
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Place : Baramati.
Date : 31/5/2012

Jayram Keshav Kashid,
Research student

Dedicated to

My Beloved Mother & Father,

Sou. Devai

&

Shri. Keshavrao Parshuram Kashid

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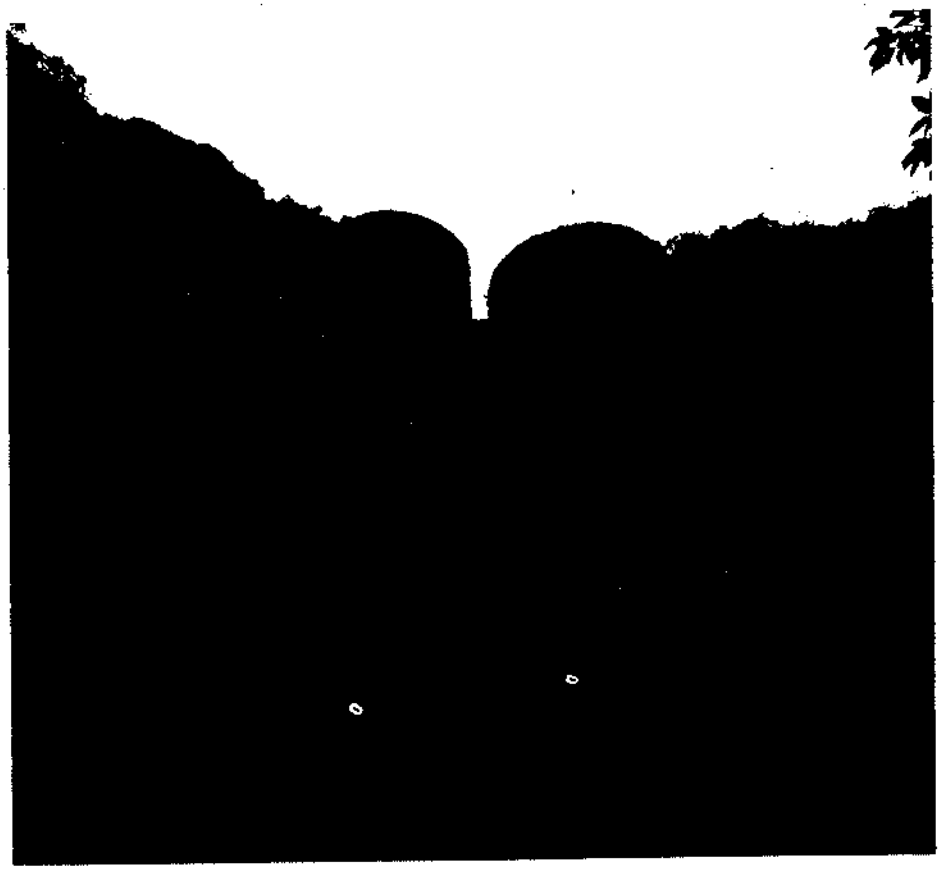
List of Abbreviations

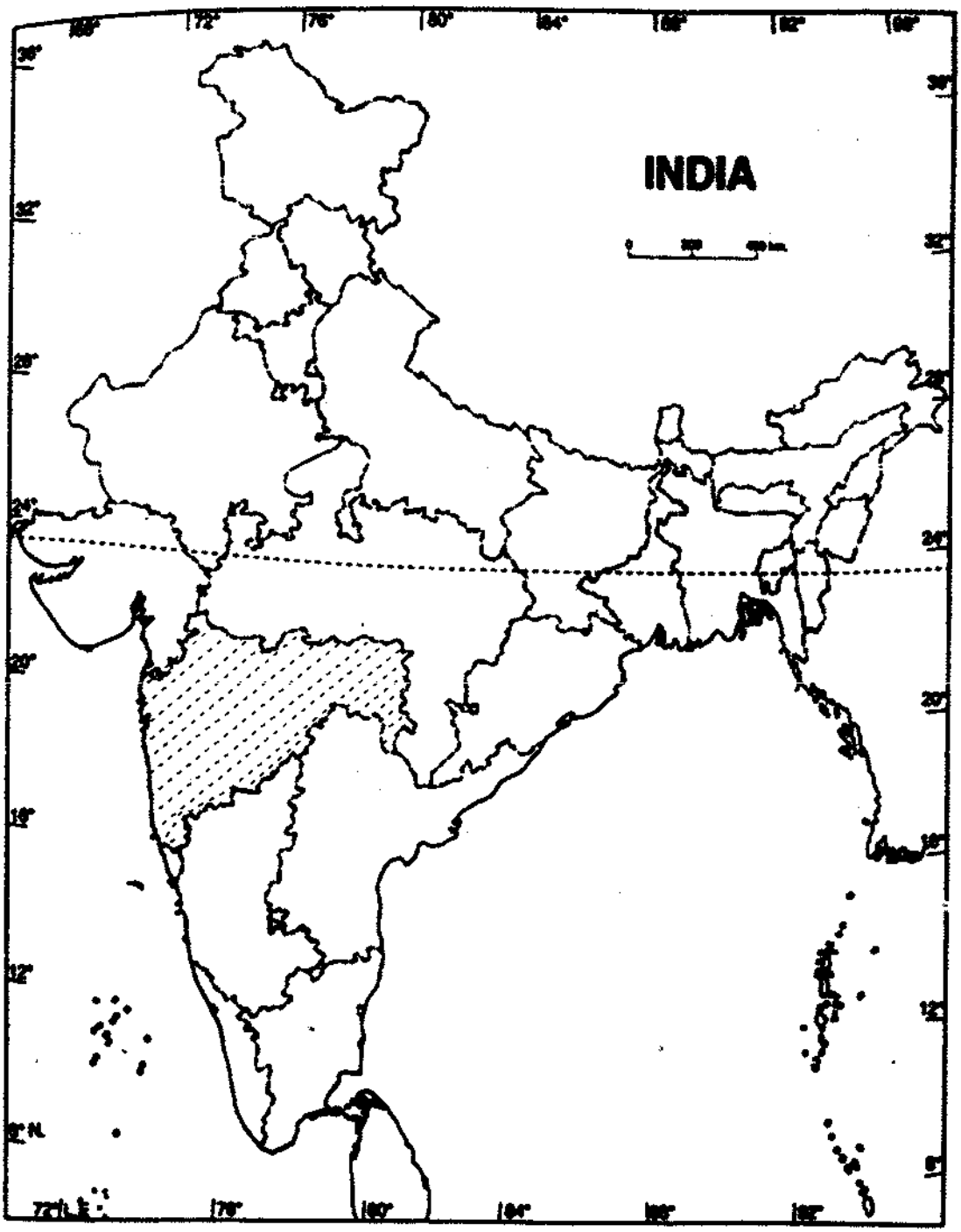
C%	-	Carbon percentage
Ca	-	Calcium
CFU	-	Colony forming units
D. W.	-	Distilled water
E. C.	-	Electric conductivity
Fig.	-	Figure
g	-	Gram
K	-	Potassium
l	-	Liter
ml	-	Milliliter
mm	-	Millimeter
N	-	Normality
g^{-1}	-	Per gram
mg^{-1}	-	Per milligram
μg^{-1}	-	Per microgram
μg^{-disc}	-	Per disc microgram
µl	-	Micro liter
sp.	-	species
%	-	Percentage
^{o}C	-	Degree Celsius
h	-	Hour(s)
min.	-	Minutes

MHA	-	Muller-Hinton agar
MHB	-	Muller-Hinton broth
MIC	-	Minimum Inhibitory Concentration
MFC	-	Minimum Fungicidal Concentration
N	-	Nitrogen
Na	-	Sodium
NaCl	-	Sodium Chloride
NCCLS	-	National Committee for Clinical Laboratory Standards
NCIM	-	National Collection of Industrial Microorganisms
P	-	Phosphorus
pH	-	Hydrogen ion concentration
PPM	-	Parts per million
PDA	-	Potato dextrose agar
r. p. m.	-	Revolution per minute
sec.	-	Second(s)
SDA	-	Saboraud's dextrose agar

1. INTRODUCTION



MAP -I



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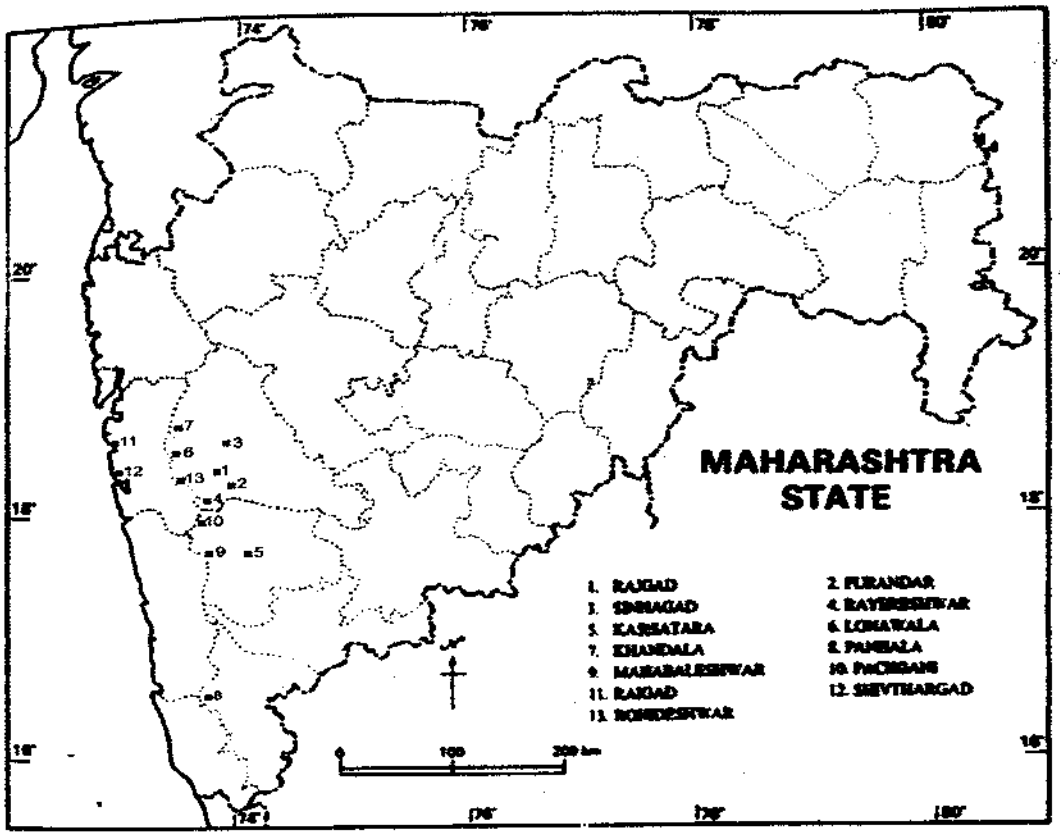

Photo Plate: Map of Western Ghats, Maharashtra.

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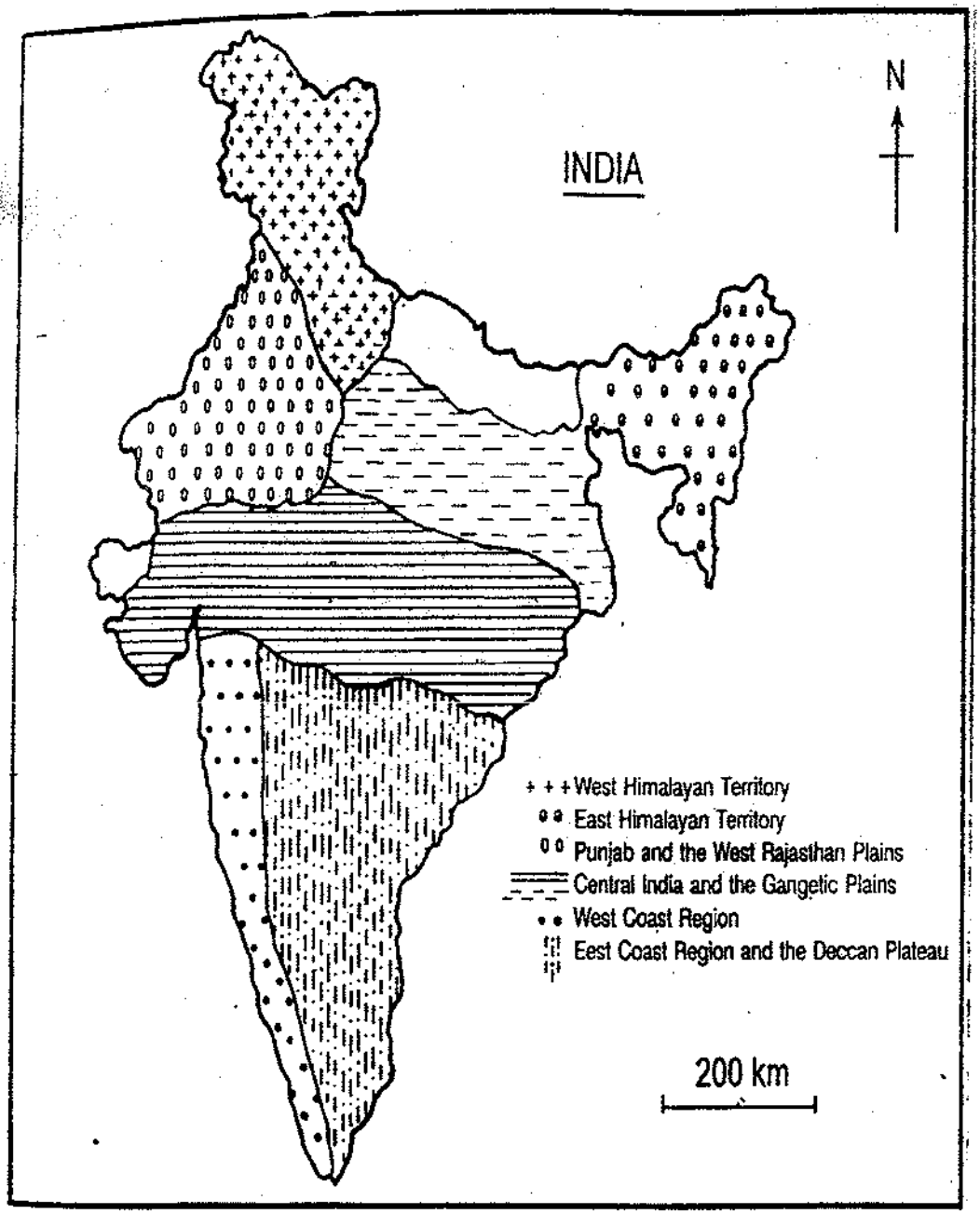


Photo Plate: Map showing bryogeographical units of Indian flora (After Pande).

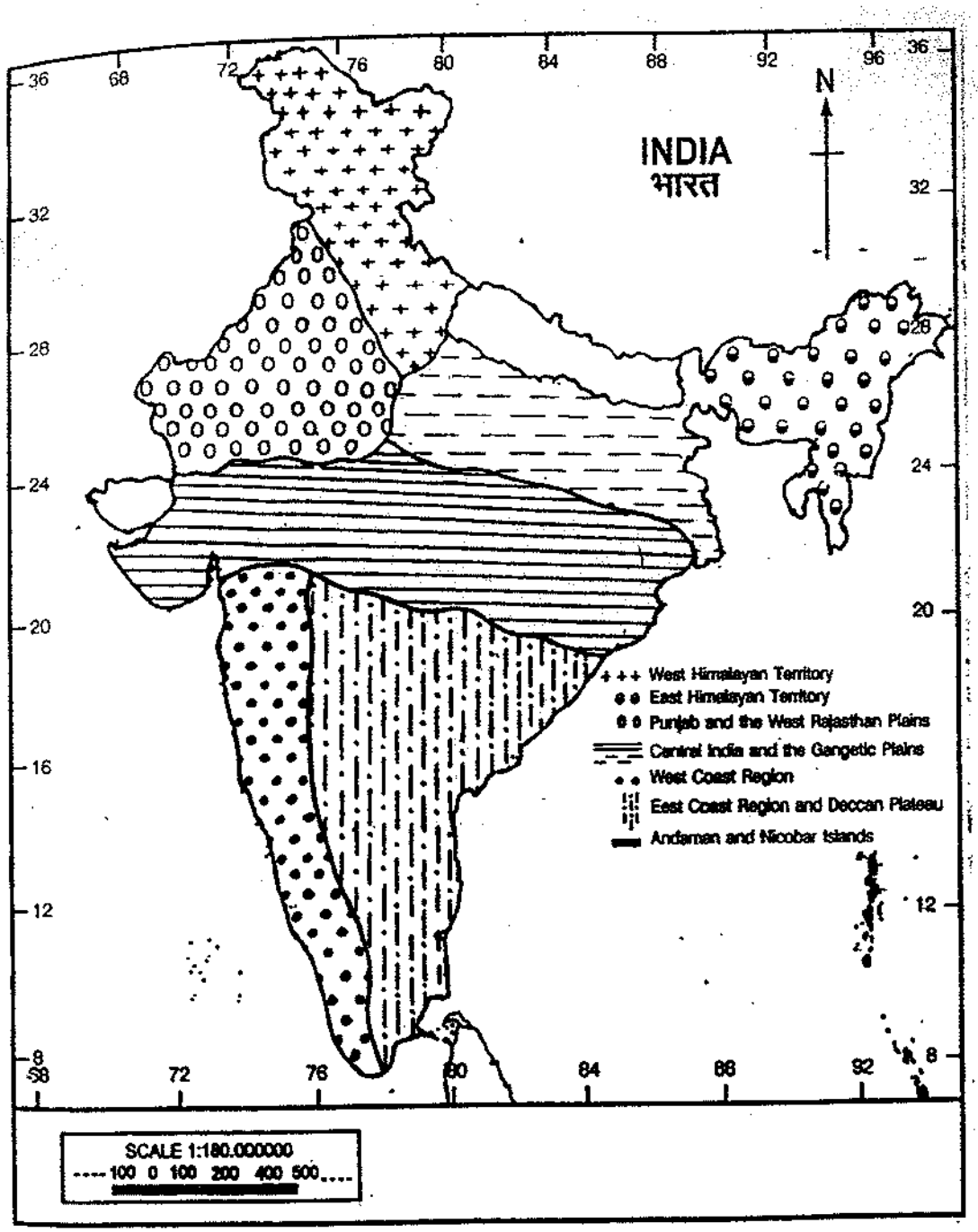


Photo Plate: Map showing bryogeographical units of Indian flora (After Joshi and Chavan, 1994).

Chapter 1. Introduction

The last two decades have witnessed a remarkable progress of bryophytes floristic studies in various parts of the World. These studies provided useful and significant data on their distribution, migration and ecology. The work done by Kashyap (1929); Kashyap and Chopra (1932a); Chopra (1938a, 1943) in India is valuable in this regard. The information of bryoflora in India is available from Muree hills (Hameed, 1942); Assam (Kachroo, 1951, 1952, 1954c, 1954e; Robinson, 1964); Gujarat (Mahabale and Chavan, 1954); South India (Rao and Udar, 1957); Mount Abu (Bapna and Vyas, 1962); Eastern Himalayas (Hattori, 1966); Nainital, Western Himalayas (Bir, 1970); Maharashtra (Joshi and Biradar, 1984) and neighbouring territories of Nepal (Banerjee, 1958; Horikawa, 1966; Grolle, 1966). Pande, (1957) in his Presidential address speaking at the annual meeting of Indian Botanical Society, Chennai (Madras) suggested to accumulate data of hepatic vegetation and study the distribution patterns and common elements of our floras with other countries and helpful in elucidating some of the interesting distribution patterns connected with other groups of plants as well.

It is also rightly dogmatized the importance of explorations by the Eminent Scientist like Santapau, (1956); Hattori, (1969) for collection, record preservation of bryophytes. With their uneventual distribution of numerous bryophyte species, many of which still are undescribed, will lost forever. Thus to say that even the Western Ghats ecosystem is being destroyed at a rapid rate without adequate conservation.

Bryophytes are classified into three Classes- Hepaticae (liverworts), Anthocerotae (hornworts) and Musci (mosses). There exists more than 24,000 species through out the world, from which 6000 species are of liverworts. These occur at plains and high mountains ranging from 4000-8000 feet's from the sea level. They flourish mostly in wet

and humid regions. Some drought enduring species grow in exposed conditions such as rocks, old stone dwellings, tree trunks and in dry heaths.

Bryophytes are taxonomically placed between algae and pteridophytes. In leafy forms, the gametophytic plant body differentiated into stem like axis and leaves. In the dominant phase thallus like plant body of bryophytes bears gametes, hence, called 'gametophyte.' The gametes are produced in complex sex organs, male 'antheridia' and female 'archegonia.' After fertilization, zygote develops into an embryo, which further by segmentation and differentiation develops sporophyte. The alternation of generation or life cycle of bryophytes shows two phases, gametophytic and sporophytic. These two generations alternate with each other in single life cycle..

The thalloid liverworts as 'hepatics' in bryophytes inhabit in damp shaded and humid localities as amphibious land dwellers. The plant body is small, green inconspicuous grow prostrate on the ground and attach to substratum by delicate, unbranched, unicellular or multicellular hair like organs called 'rhizoids.' Unlike most of the higher plants, bryophytes are not found as single individuals but in groups of individuals. Depending upon the species, genus and family, each individual has a genetically fixed method of ramification, may be called its 'growth form.' The assemblage of individuals and growth forms modified by external factors, together provides the characteristics which can be termed as 'life form' (Warming, 1896).

Research dealing with life forms in bryophytes is meager (Richards, 1932) and it includes the important life forms of the hepatics (Geisenhagen, 1910; Herzog, 1916). In the following paragraphs, we have tried to characterize the important life forms on the basis of our observations are as -

1. Annuals - These are limited to open mineral soils. The gametophyte stops growing once it has produced gametangia and dies after the sporangium has ripened. These are often pioneer mosses on open mineral rich soils, along with liverworts like *Riccia* and *Cyathodium*.

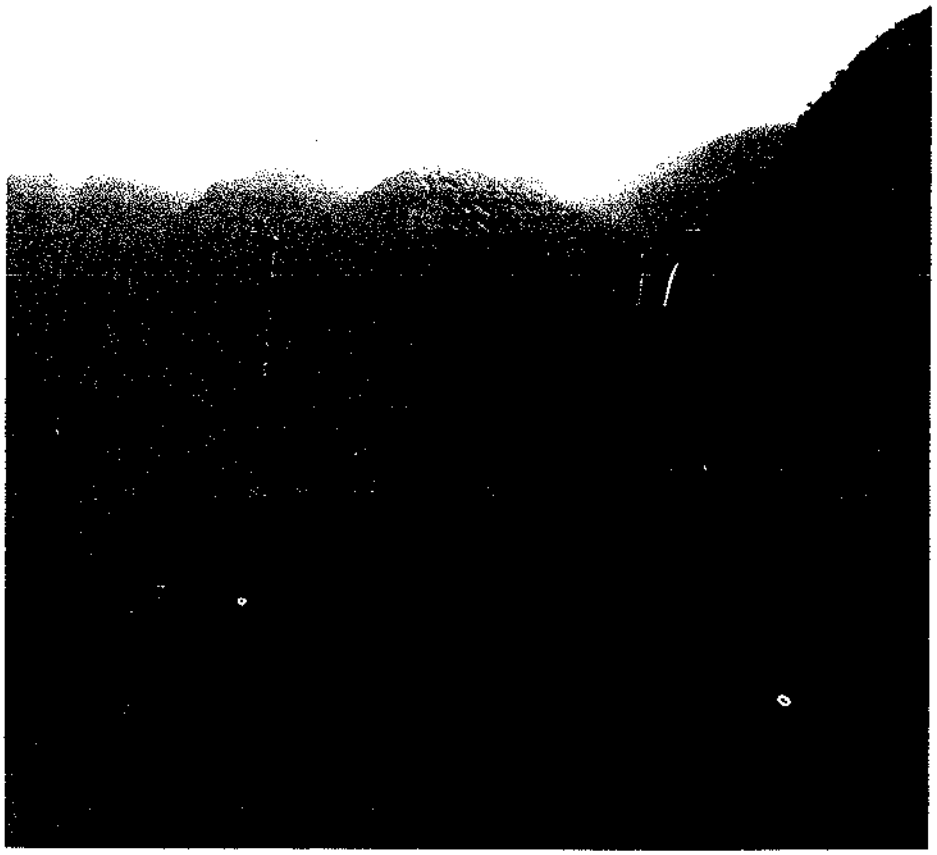
2. Pendants - The life forms closely connected with two environmental factors as light and water. Mostly species of *Plagiochasma*, *Targionia* are a sticking decoration in humid forests.
3. Fans - Creeping liverwort species of *Cyathodium* growing on a vertical base (trees and rocks), the shoots of which branch towards one another in the same plain, projecting horizontally to obliquely downwards and usually have flattened thallus.
4. Mats - The rhizoids of which often form special organ of attachment, they grow on shady wet walls and the main shoots of which lie close to the substrate and are attached to it by rhizoids. Ex. *Fossombronia* and *Sewardiella*.

Keeping view of this work it was thought worthwhile to make a critical survey of hepatic vegetation from Western Ghats of Maharashtra (Photo Plate Map- II) and accordingly an extensive collection was made during months of rainy season i.e. July, 2008 to September, 2011. After the exploration at various localities, the richness of species had been revealed qualitatively and quantitatively. This is a crucial survey and considering the time spent by us, on account of technical constrains, natural hazards etc. In this area of collection, the prospect of a better harvest should not be left uncared for.

The present study provides the first hand consolidated account of thalloid hepatics including 11 species belonging to eight genera, distributed over five families. The sequential placement of the families and genera are according to Schuster's system of classification (1958c, 1979). This study is entirely based on the material collected during three visits to the various localities of Western Ghats of Maharashtra. The detailed illustrations were done for Physico-chemical and biological characteristics of liverworts associated soil, antimicrobial properties of extracts and characterization of selected thalloids. The pertinent literature related to work has been consulted from Jaykar library, University of Pune, Pune, library; websites, Agharkar Research Institute, Pune; and personal library of my research guide Dr. Chavan. The map of India and Maharashtra

Maharashtra showing the localities of collection have been adopted from Readers Digest 'Great World Atlas' and are based upon Survey of India Map.

2. REVIEW OF LITERATURE



Chapter 2. Review of literature

History of Bryophytes:

The word 'bryophyta' was coined and used for the first time by Robert Brown and the rank of division to this group bryophyta was first given by Schimper and Howe. It is divided into three Classes as - Hapaticae, Anthocerotae and Musci. Rothmaler have suggested the following class taxons for the old one as Heapaticopsida for Hepaticae, Anthocerotopsida for Anthocerotae and Bryopsida for Musci as recognized by ICBN.

An account of Hepaticae:

The estimated number of liverworts species are 6000-8000. On basis of habitat, the liverworts grow as terrestrial, aquatic, lithophytes and epiphytes. Liverworts are green, dorsiventral, thalloid and foliose. The rhizoids are smooth walled as well as tubericulated, growing cells protected by scales. Sex organs develop from superficial cell on dorsal side. Sporophyte differentiate into foot, seta and capsule. Gametophyte has central axis and leafy expansions but has no midrib. Archegonia is in linear rows. Sporogonium is small, has no meristematic tissue, columella absent.

Bryology in India :

Due to phytoclimatic variation, the seven biogeographically regions occurs as Western Himalaya, the Punjab and the West Rajasthan, the Gangetic plains, the Central India, the Western and Eastern Ghats, the Deccan Plateau and the Islands of Andaman and Nicobar (Joshi *et al.*, 1992). Griffith, (1842) recorded the number of mosses and liverworts from Khasi hills in Assam under private study and published the valuable notes 'Notulae and plantae Asiaticae' is regarded as the first notable contribution to Indian bryology.

The studies on the Indian orient :

Studies in the liverworts of Mt. Abu by Bapna and Vyas (1962); Nainital by Bir, (1970); Indian hepatics by Chopra, R. S. (1938a, 1943); Indian orient by Stephani, (1900-1924); a monograph on liverworts of Western Himalayas and Punjab Plains by Kashyap, (1929); Indian Anthocerotaceae by Bhardwaj (1948); Indian hepaticae by Pande and Misra (1943a); hepatic vegetation by Panchmarhi (Madhya Pradesh) by Pande and Shrivastava, (1952); liverworts from Yere, South India by Rao and Udar, (1957); collection of bryophytes from upper Assam by Robinson, (1964); the 'botanical exploration of India' a book published by Santapau, (1956); bryology in India by Udar (1976); recent concept in the taxonomy of *Notothylas* by Udar and Singh, (1981) (a book published by J. Cramer (1991) at Berlin). Three mosses record from Andaman Islands by Rajesh, (2010). Liverwort flora of Andaman Islands by Joshi *et al.*, (1989, 1991); Joshi and Chavan, (1994); Joshi, (2001); liverworts in Gujarat by Mahabale and Chavan (1954); Indian hornworts, a taxonomic study by Asthana and Srivastava, (1991) Nair and Madhusoodanan, (2002) have to pertain knowledge from different localities of India. Information on Indian hepatics reported (Bapna and Kachroo, 2000). Biodiversity in Indian Garhwal Himalaya reported by Negi and Gadgil, (2002). Studies on the liverworts from Khasi and Jaintia hills of Meghalaya carried out by Singh, (2002). Liverworts *Riccia himalayensis* and *R. discolor* reported from both West and East Himalayas as well as from plains of Assam, West Bengal, Madaya Pradesh and South India (Mondal, 2007). Study on liverworts from Meghalaya (India) and Eastern Himalayas are reported by Singh and Singh, (2008). Pocs and coworkers (2007) have reported thirty three hepatic taxa from Western Ghats of Kerala state. Checklist from Kerala State of India are reported by Manju *et al.*, (2008). Bryoflora of Baphlamalai hill in Eastern Ghats of Orissa, India, was reported by ENVIS, Government of India (2009). Studies on genera *Riccia* L. from West Bengal reported by Singh *et al.*, (2010). The details of type and checklist of the bryophytes from Tamil Nadu state documented by Daniel, (2010). Some

taxa of mosses from Chandigad state has been studied by Kaur *et al.*, (2010). Some bryophytes recorded from Andaman Islands, India by Rajesh, (2010). This mentioned bryophytic study is regarding taxonomy.

Hepatics from Western Ghats, Maharashtra:

The limited biosystematics literature dealing with Western Ghats bryoflora is recorded. The floristic outline is well known and provides the basis for the production of modern regional floras. Studies on the liverworts flora from Western Ghats with special reference to Maharashtra, India carried out by Joshi and Biradar, (1984).

The taxonomic contribution in mosses of Mahabaleshwar received from Dabhade (1974); Joshi and Biradar (1984) and Ulka and Karadge (2010).

The Importance of Bryophytes :

Belkin *et al.*, (1952) found that *Polytrichum juniperium* extracts had anticancer activity against Sarcoma in mice. Bryophytes have brought the attention of several workers. *Bryum* species and their paste can apply on poultice and to reduce the pain of burns (Flowers, 1957). Antibiotic compounds isolate from many bryophytes (McCleary *et al.*, 1960).

Antibiotic properties :

Chemical substance or bioactive compound in small concentrations are capable of inhabiting the growth of other organisms.

McCleary and Walkington, (1966) tested antibiotic activities of fifty species of mosses against two bacteria *Gaffkeya tetragena* and *Staphylococcus aureus* and suggested that the non-ionized organic acids and polyphenolic compounds might contribute to the antibiotic properties. *Barbula* and *Timella* petroleum extract showed an antibiotic activity against thirty three bacterial strains, and found the high occurrence of antibacterial activity in extracts of *Barbula* species (Gupta and Singh, 1971). Hartwell, (1971) has reported antitumor properties of *Marchantia polymorpha*, and *polytrichum*

communae extracts. *Polytrichum communae* helps to dissolve stones of kidney and gall bladder (Gulabani, 1974). Isolated diplophyllin from *Diplophyllum* showed significant activity against Carcinoma (Ohta *et al.*, 1977). Antibacterial properties of *Fossombronia himalayensis* Kash. extracts tested against against organisms including two gram positive and two gram negative bacteria *viz. Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae*, and *Bacillus substilis*, respectively (Joshi and Desai, 1988). *Pallavicinia canaras* Gray. dimethylsulphoxide (DMSO) organic fractions and distilled water fraction against tested organisms (Deshpande and Joshi, 1990). Antibacterial activity of *Reboulia hemispherica* (L.) Reddi. extracts from south India tested against same bacterial strains with positive response of both DMSO and aqueous fractions. There is an evidence in literature that confirm the antibiotic activity in *Lunularia*, *Rhynchostegium* and *Pleurochaete* against bacterial and fungal organisms (Basile, 1998, 1998_a, 1998_b). A very few reports are available on antibiotic activity from North India (Kaushik *et al.*, 2000; Sudarshan Kumar, 2007).

Asakawa *et al.*, (1982) have isolated three prenyl bibenzyls from *Radula* sp. and demonstrated that they could inhibit growth of *Staphylococcus aureus. Rhodobryum gingatium* and *R. roseum* used to treat cardiovascular diseases and nervous prostration. *Haplocladium microphyllum* to treat bronchitis and tonsillitis. A mixture of *Coenocephalum conicum* and *Marchancia polymorpha* with vegetable oil is used on bites, boils, burns and wounds (Ando, 1983). In Himalaya, a mixture of moss ash with fat and honey is used as soothing and healing ointment for cuts, burns and wounds (Pant *et al.*, 1986). Scanning electron microscopic studies on liverworts carried out (Kumar *et al.*, 1987).

Medicinal Importance :

Long ago Chinese, Europeans and North Americans have used bryophytes as 'medicines.' Number of secondary metabolites in bryophytes make important value. Liverwort *Polytrichum communae* extract used for reducing inflammation and fever

(Hu, 1987). Numerous compounds like oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, prenylquinones, aromatic and phenolic compounds reported from bryophytes (Pant and Tewari, 1990).

Ecological Importance :

Vegetative Fragments of bryophytes could help to prevent erosion. Lichens and mosses play an important role in soil formation. Thalli secrete organic acids which gradually dissolve and distingrate the rocks and amount of fertile soil gathers in the crevices of rock surfaces. It plays a remarkable role as 'rock builders.' Insoluble calcium carbonate precipitates and harden forming calcareous (lime) rocklike deposites around these plants.

Bryophytes species serve as bioindicator for specific minerals. Liverwort *Jungermannia vulcanicola* and mosses *Sphagnum* and *Polytrichum* play active role in deposition of iron ores. *Ceratodon purpureus* suggest the high amount of nitrogen, whereas *Pogonatum apinium* signals less nitrogen. *Polytrichum* is a good acid indicator by Crum (1973). Also, bryophytes are good indicator of soil pH. Some species are used as soil conditioner in agriculture. Peat added into heavy soil and improve the texture with preventing of cracking. *Sphagnum* is a good methane producer as a 'bog moss' in acidic ponds. Also it is used as a filtering and adsorbing agent for treatment of waste water, effluents of factories containing toxic discharge. Birds employ the fragments of gametophores of mosses, and liverworts, in construction of nests. (Glime, 2007).

Chemosystematics of liverworts:

As India is one of the 12 mega diversity country in the world (Nayar, 1996). Due to lack of commercial value and inconspicuous nature of bryophytic plants, very few people know about their therapeutic uses. They are used by human being as well as vertebrates and invertebrates (Manju *et al.*, 2009). The literature on therapeutic use of

bryophytes are documented in China, Europe, North America, Japan, Taiwan, New Zealand, Madagascar, Nepal, Pakistan, and in India, with respect to their chemistry and drug pharmacology. Asakawa *et al.*, (1991) noted the presence of secondary metabolites, have very much importance and review about the chemistry of bryophytes have been published every few years. Wide variety of structurally interesting compounds having antibacterial, antifungal, anticancer and diuretic activity have been reported (Asakawa, 1995). Scientists estimated that a quarter of the total plant genes (20,000 to 60,000) are responsible for encoding enzymes for secondary metabolites (Pichersky and Gang, 2000). Liverworts have many oil bodies that contain large quantities of terpenoids, whereas, less terpenoids are found in mosses because of absence of oil bodies Secondary metabolites may change due to exposure to light or heat during the storage (Asakawa, 2001).

Bioactive compounds from bryophytes:

Different parts of plant may contain different compounds. Plants are still an invaluable reservoir for novel bioactive natural products. With the beginning involved studies of bioactive compounds which are readily available from natural sources.

Several unsaturated lipids, fatty acid esters, sesquiterpenes and sesquiterpenoids, flavonoids, tri-terpenoids, phenolics, and other chemical substances are reported to be present in bryophytes. The sesquiterpenes and sesqui-terpenoids show an antitumor, cytotoxic, and phytotoxic activities, and cause allergic contact dermatitis in humans. Sesquiterpene lactones provide resistance to insect feeding. Few lactones possess antibacterial, antifungal and anti-helminthic properties. The substances show an antitumor, cytotoxic, antimicrobial and phytotoxic activities and cause allergic contact dermatitis in humans. The tri-terpenoids and steroids play an important role in therapeutic uses for cardio tonics, anti-inflammatory agent and anabolic agent. Tri-terpenoid saponins demonstrat antifungal, cancer static, anti-ulcergenic, sedative, spermicidal activity. In many developing countries, about eighty percent of available therapeutic

substances are originated from medicinal plants (Keles *et al.*, 2001). The plants can provide nutrients and niche for the microbes. Terrestrial microorganisms in associated soil of thalloid liverworts are another important source of novel natural products, mainly secondary metabolites, having antibiotic activity. These compounds has antimicrobial activity due to presence of bioactive compounds like quinine, artemisinin, cryptolepine as well as anticancer compounds such as camptothecin, taxol and austocytin, namenamecin, lomaiviticins, pyrrole alkaloids, and hemiasterlins (Ireland *et al.*, 2003).

During last decades several unsaturated lipids, fatty acid esters, flavonoids, triterpenoids, phenolics and other chemical substances are reported to be present in bryophytes. Less than 10% of bryophytes chemistry are studied and different types of terpenoids, bisbibenzyls, flavonoids, alkaloids and other novel compounds have been isolated and documented in regular review (Zinsmeister *et al.*, 1991; Asakawa, 2004). Liverworts are the best known for their rich chemistry than mosses. Although, mosses also produce secondary metabolites like flavonoids and biflavonoid. Beside the flavonoids, mosses contain some simple phenolic compounds. e.g. sphagnorubins from *Sphagnum* species, ohioensins from *Polytrichum* species. Among the secondary metabolites isolated from mosses, biflavonoids are the characteristic compounds. Bioactive compounds in small concentrations are capable of inhabiting the growth of other organisms and shown antagonistic effect which are responsible for the suppression of another type organisms.

Bioactive compounds from thalloid liverworts :

Due to tininess and occurance in remote places, phytochemistry of liverworts is neglected for the long time. Though, these have no nutritional value for human, more than 700 terpenoids and 220 aromatic compounds are isolated from Hepaticae (Maxwell and Ramprasad, 1988). However, flavonoids and other phenolic compounds are also frequently produced.

Monoterpenoids are known for the distinctive smell that many liverworts produce when crushed. Among approximately 60 monoterpenoids found in liverworts, mainly alpha-pinene, beta-pinene and limonene are the most abundant compounds Bis-bibenzyls are new class of natural products that is restricted to the liverworts (Ali and Kondo, 1992).

Among the monoterpenoids, sesquiterpenoids are more abundant in liverworts. More than 500 sesquiterpenoids are isolated from Hepaticae so far. The tri-terpenoid are saponins demonstrated as antifungal, anti-inflammatory, cancer static, anti-ulcergenic, sedative, spermicidal, antitrombic and corticosteroid activity. Steroids play an important role in development and control of reproductive tract in human being (progesterone and testosterone). Steroids have wide range of therapeutic applications as cardio tonics (digitoxin), vitamin D precursor (ergosterol), anti-inflammatory agent (corticosteroids) and anabolic agents (androgen) (Maxwell and Ramprasad, 1988). Liverworts species and their chemistry has been extensively studied and a variety of secondary metabolites are isolated. Secondary metabolites are often able to contribute to the organisms survival by defend itself. Plants defend themselves against insect herbivores through toxic compounds like terpenoids, alkaloids etc. (Harborne, 2001). The most of the widespread sesquterpene in liverworts is *ent*-bicyclogermacrene and eudesmanes. These substances have antimicrobial activities. Sesquitèrpene lactones provide resistance to an insect feeding. Liverworts are rich source of diterpenoids. More than 200 diterpenoids, representing about 20 carbon skeleton, have been found in the Hepaticae. Among them clerodanes, labdanes and kauranes are the most widespread type. Secondary metabolites can be divided into several structure classes, terpenoids, alkaloids, polyketides, non ribosomal peptides, phenolics based on their biosynthetic pathway. Bibenzyls and their di- and tetramers are frequently produced. Terpenoids are considered to be the characteristic metabolite of liverworts (Xiaowei, 2007).

It is reported that unique odour results from combination of compounds including monoterpenes hydrocarbons, fatty acids and methyl esters (Hayashi *et al.*, 1977). The moss *Brychithecium procumbens* and liverworts *Asterella sanguinea* and *Paleacea* have shown the broadest spectrum of antibiotic activity. These are 'remarkable reservoir' of new natural products or secondary compounds, many of which have shown interesting biological activities, include antimicrobial, cytotoxic, antitumor, vasopressin antagonist, cardiotonic, allergy causing, irritancy and tumor affecting, insect antifeedant, insecticidal, molluscidal, pesticidal, plant growth regulatory, superoxide anion radical release inhibition and 5-lipoxygenase, calmodulin, hyaluronidase and cyclo-oxygenase inhibition features (Banerjee and Sen, 1979). A variety of secondary metabolites have been isolated from liverworts. Terpenoids are considered as the characteristic prominent metabolite of liverworts (Asakawa, 1982). This sensitivity to change in climatic conditions make bryophytes a valuable indicator of forest integrity (Richards, 1984). Bryophytes known for their use in ethnobotany and are subjected to cure disease, threat to plants and animals or in the household (Ando and Matsuo, 1984). The bioactive compounds extracted from bryophytes have also shown antitumor activity (Castaldo Cobianchi *et al.*, 1988). This group also contains numerous potential compounds like oligo, polysaccharides, sugar alcohols, amino acids, phenylquinones, and phenolic compounds (Pant and Tewari, 1990). They contain numerous bioactive compounds. The biologically active substances found in liverworts with respect to their chemistry, pharmacology and application as source of cosmetics and medicinal or agricultural drugs (Asakawa, 1993). There are several reports about role of bryophytes as medicinal sources for more than 400 years in China (Asakawa, 1994). Researchers are interested in finding new bioactive compounds with therapeutic uses. The sources are found in all ecosystems, the biomass productivities. Bioactive compounds can vary in each system Nature of terpenoids and aromatic compounds depend on the type of environment in which liverworts normally

live (Asakawa, 1995). Among the bryophytes, the liverworts are best known for their rich source of secondary metabolites.

Liverworts phytochemistry has been studied in detail as they possess oil bodies. Bryophytes possess extremely high amount of terpenoids, phenolics (flavonoids and bibenzyls derivatives), glycosides, fatty acids and some rare aromatic compounds. Components like beta-phellandrene, peculiaroxide, bicyclogermacrene, spathulenol, and fusicoccadiene are observed in the extract of liverworts (Asakawa, 1995). The phytochemical studies have shown that they contain a wide variety of structurally interesting compounds, which are antibacterial, antifungal, anticancer and diuretic (Asakawa, 1995). Active sesquiterpenes are neverthless isolated from two species of liverworts *Porella cordeana* and *Chiloscyphus rivularis* (Chongming *et al.*, 1997). Infectious diseases are caused by bacterial organisms. These compounds enhance the resistance. Thus, there is a corresponding rise in the universal demand of natural therapeutics (Basile, 1998a). Liverworts phytochemistry has been studied in detail because they possess oil bodies. The bryophytes form an important component of the forest ecosystem in India (Srinivasan, 1998). They may attribute to prevent Colon cancer (Atlah *et al.*, 1999).

Liverworts are characterized by the presence of oil bodies in which terpenoids and aromatic compounds are accumulated (Flegel and Becker, 2000). Terpenoids, phenolics and volatile constituents have been investigated in some bryophytes (Saritas *et al.*, 2001). A literature search revealed no records on the antifungal activity of Indian bryophytes. In other countries study indicates that bryophytes are a rich source of antifungal agents. The Chinese, Europeans, and North Americans have used bryophytes as medicine for hundreds of years. Several hundreds of new compounds have been isolated from bryophytes and their structure elucidated (Asakawa, 2001). The World Health Organization (WHO) estimated that, 80% of the population of developing countries relies

on traditional medicines, mostly plant drugs, for their primary health care needs (Heinrich and Gibbons, 2001). These are occur in natural forms at the high altitudinal areas and many have potential medicinal properties. It is known that drug resistance develops in human pathogens against commonly used antibiotics. Recently exploration yielded about 250 species of bryophytes in Kerala state (Manju and Madhusoodanan, 2002).

Liverworts are characterized by the presence of oil bodies in which terpenoids and aromatic compounds present (Flegel and Becker, 2000). In ancient times, people used plants to cure disease, based on experience and accumulated as traditional medicine. From the onset of the organic chemistry, studies of bioactive compounds available from natural environment have been undertaken. In cancer and infectious diseases, over 75% of drugs are natural products or have natural origins (Newman *et al.*, 2003). The substances from mosses resist to fungal attack (Saxena and Harinder, 2004). However, bibenzyls and di-, tetramers also frequently also produced. *Scapantia* species produce various kinds of sesquiterpenoids which are ubiquinons in other liverworts (Asakawa, 2004). Abu-Shanab *et al.*, (2004) studied the screening of plant extracts for their antimicrobial activity as well as to discover new antimicrobial compounds. The *Bazzania* species are known for rich source of many kinds of sesquiterpenoids. The alpha-prenyl group structures are widely found in natural sources and often show an interesting biological activity (Asakawa, 2004). The seco-eudesmane compound have both cytotoxic and antibacterial activity (Kumar *et al.*, 2005). These are used in the ethno medicinal field from times immemorial in many parts of the World (Subhisha and Subramanian, 2005). It has been shown that, mosses rich in flavonoid possess strong antimicrobial activity. Antimicrobial activity of methanol extract of *Hypnum cupressiformae* was also analyzed (Dulger *et al.*, 2005). Compounds like terpenoids, phenolics, volatile constituents, flavonoids, isoflavonoids, and biflavonoids have been reported as possible chemical barriers against growth of microorganisms. The liverwort *Palustriella comumutata* (Hedw.) reported to possess a novel antimicrobial molecules which affect

gram negative and gram positive bacteria (Semra *et al.*, 2006). The fact that biochemicals with antibiotic, neurotropic, insect antifeedent and antitumor activity have been isolated from various species adds further weight to the argument for maintaining and exploring bryophyte germplasm (Spjut *et al.*, 2006). It has been demonstrated that most of the liverworts contain mainly sesqui-terpenoids, acetogenins, and lipophilic aromatic compounds like bibenzyls, long chain phenols, phthalides, isocaumarine which show biological activities (Asakawa, 2007). Antimicrobial activity of some Indian mosses (Rawat and Govindarajan, 2007). However, the chemistry of bryophytes is poorly known and results are very scattered (Sabovljevic and Sabovljevic, 2008). Also, Chilean native moss *Sphagnum magellanicum* has been reported for antimicrobial properties (Montenegro *et al.*, 2009). Ergin and Barbaros, (2009) reported the antimicrobial activity of *Thuidium delicatum* extracts. Liverworts have ability to form intracellular oil bodies, containing a wide variety of terpenoids and aromatic compounds. Veljic *et al.*, (2009) have reported the antimicrobial activity of methanol extracts of mosses from Serbia. Volatile compositions of mosses have been studied (Li and Zhao, 2009; Cansu *et al.*, 2010).

Need of study:

The state of Maharashtra is one of the important location of biodiversity in Western Ghats belt, located in the Central Peninsular India. It has remarkable physical homogeneity, enforced by its underlying geology. The Sahyadri range is the physical backbone of Maharashtra, rising on an average to an elevation of 1000 m. Sahyadri range is narrow coastal lowland, barely 50 km wide. It has a Western coastline stretching 330 miles (530 Km) along the Arabian Sea from Goa. The Maharashtra State is blessed with humid tropical climate as an annual rainfall vary from 400-6000 mm, and comes for 3-4 months in an year and a varied topography, and wide diversity of habitats including bryoflora. Maharashtra has typical monsoon climate with hot, rainy and cold weather seasons. During April and May thunderstorms are common all over the state.

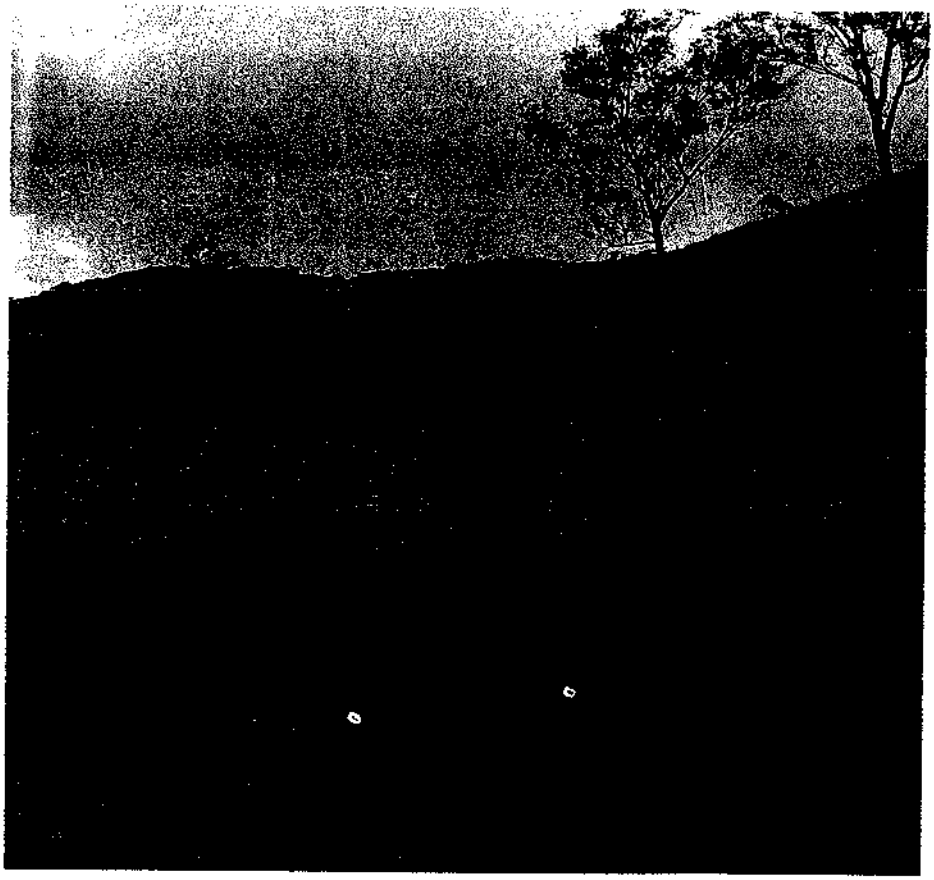
Temperature varies between 22°C-39°C during this season. July is the wettest month, while August too gets substantial rain. Monsoon starts its retreat with the mid of September from the state. Cool dry spell, with clear skies gentle breeze and pleasant weather prevails from November to February. Rainfall differs from region to region. Rainfall particularly concentrates to the Konkan and Sahyadrian Maharashtra. Central Maharashtra receives less rainfall. However, under the influence of the Bay of Bengal, eastern Vidarbha receives good rainfall in July, August and September.

Aims and Objectives of the present study:

The taxonomic study of bryophytes from Western Ghats of Maharashtra was carried out in the past (Joshi and Biradar, 1984). As these plants are therapeutically important, therefore, the concentration is made on it specially with reference to antibiotic activity.

The present work was undertaken to understand and enhance the knowledge of bellow ground biodiversity and their role with special interest to fungi from different localities of Western Ghats, Maharashtra, India. The surveying and collection of thalloid liverworts from different localities includes such as Purandar, Rajgad, Sinhagad, Rayereshwar, Kas;Satara, Lonawala, Khandala, Panhala, Mahabaleshwar, Pachgani, Shivthargad, Raigad, and Rohideshwar. The purpose of this study is to determine analysis of associated soil for physico-chemical and biological characteristics, antimicrobial screening of extracts and characterization by effect of different parameters.

3. MATERIAL AND METHODS



Material and methods:

A. Surveying.

B. Collection and Identification of thalloid liverworts.

C. Physico-chemical characteristics of associated soil.

D. Biological characteristics of associated soil.

E. Antimicrobial screening of thalloid liverworts.

Chapter 3. Material and Methods

A. Surveying of bryophytes at ecologically different altitudinal areas of Western Ghats, Maharashtra:

Maharashtra, the third largest state of India, both in area and population. It has western coastline stretching 330 miles (530 Km) along the Arabian Sea from Goa. The Western Ghats are not true mountains but, are the faulted edge of the Deccan Plateau. They are believed to have been formed during the breakup of the super continent of Gondwana, some 150 million years ago. Its length is about 1,600 km, N–S and width 100 km, E–W. Climate in the Western Ghats varies with altitudinal gradation and distance from the equator. The climate is humid and tropical in the lower reaches tempered by proximity to the sea. Elevations of 1,500 m (4,921 ft) and above in the north and 2,000 m (6,562 ft) and above in the south have a more temperate climate. Average annual temperature are around 15 °C. In some parts, frost is common and temperatures touch the freezing point during the winter months. Mean temperature range from 20 °C (68 °F) in the south and 24 °C (75 °F) in the north. It has also been observed that the coldest period in south Western Ghats coincide with the wettest. Number of forts are situated in Western Ghats of Maharashtra and these are surveyed for our study. The Western Ghats extend from the Satpura range in north boundry of Maharashtra to south past Goa, through Karnataka, Kerala and into Tamil Nadu. The major hill range starting from north is the *Sahyadhri* range.

The Rajgad is King of forts located at 18°14'45"N, 73°40'58"E and 42 km to the south west of Pune, about 15-16 km west of Nasrapur in the Sahyadris range, fort is approximately 4250 feet above sea level (Pl. I-A).

Purandar fort located at 18°16'50"N, 73°58'27"E stands 4,472 ft. above the sea 1,387 m (Pl. I-B). It actually consists of two forts - Purandar and Vajragad. The relative

humidity is normally high during mansoon which favours growth of bryophyte plants in such area.

Sinhagad fort is located roughly 30 kilometers southwest of the city Pune (Pl. I-C). Previously called 'Kondana' was also strategically located at the center of a string of other forts such as Rajgad, Purandar and Torna. The fort is located at 18°21'56"N 73°45'20"E, 18.365604°N 73.755555°E in Sahyadri mountains on a deserted cliff of Bhuleswar range at a height of 1350 m above the sea level.

Kas; Satara region has geographically located at 17°42'0"N, 73°50'E (Pl. II-B). Lonawala lie at 18°44'59"N, 73°25'2"E and 5 km, apart on the western slopes of Sahyadris, straddling the Mumbai-Pune highway at an altitude of 625 m (Pl. II-C). Temperatures vary from 12 °C in winter to around 36 °C at the height of summer. The annual rainfall averages 450 cms.

Its other famous cousin town Khandala lies just 5 Km away (Pl. III-A). Although is comparatively small, it is equally blessed with natural beauty and bounty. Deep valley on one side and high hills on the other side divide Khandala and Lonawala. It is located at 17° 42' 0" N, 73° 50' 0" E.

Panhala is located at 16°49'12"N 74°7'12"E, 16.82°N 74.12°E. It has an average elevation of 754 m (2473 ft). The fort is at 20 km northwest of Kolhapur and is the largest of all Deccan forts (Pl. III-B).

Mahabaleshwar is located at 17°55'N 73°40'E, 17.92°N 73.67°E. It has an average elevation of 1,353 meter's (4,439 ft). It is located about 120 km southwest of Pune, and 285 km from Bombay and a vast plateau measuring 150 km^2 and bound by valleys on all sides (Pl. III-C). It reaches a height of 1,438 m at its highest peak above sea level known as 'Wilson Point.'

Rohida fort is located to the south of Bhor. It is popularly known as 'Vichitragad,' and 57 K. m. away from Pune, and lies 3621 ft. above sea level. It is located at 18°6'9"N, 73°49'17"E.

Raigad is a hill fortress situated in Raigad district of Maharashtra. The fort, which rises 2,700 ft. above sea level, is located in the Sahyadri mountain ranges, near from Bhor. It is located at 18 14' N 73 26' E.

B. Collection of thalloid liverworts:

The present study is based on the material collected from various localities such as as Rajgad, Purandar, Sinhagad, Rayreshwar, Kas;Satara, Lonawala, Khandala, Panhala, Mahabaleshwar, Pachgani, Shivathargad, Raigad and Rohideshwar regions of Western Ghats of Maharashtra, India. The material was collected during July, 2008 to September, 2011 from shady places along the sides of graveled foot paths, near crack crevices, wet walls, tree trunks, semi exposed substratum along with rainy season herbaceous plants.

A part of the collected taxa was also preserved dry in brown paper packets. However, for the microscopic examination, the same was subjected to various treatments to facilitate a better study. After proper cleaning and dressing of taxa, investigation from various angles are carried out, which resulted in proper identification of the same. It was confirmed by comparing with the type specimen and literature.

The preserved specimens along with necessary details are maintained in the Bryological Herbarium, Department of Botany, Tuljaram Chaturchand College, Baramati, Dist. Pune- 413 102, Maharashtra, India.

C. Physico-chemical characteristics of liverworts associated soil:

The Physico-chemical characteristics of thalloid liverworts associated soil from localities of Western Ghats of Maharashtra was carried out. It contains both organic compounds like available nitrogen (N), and inorganic compounds like Ca, Mg, Na, K, Fe, Al, P, S, Cl etc.

Soil sampling and sample preparation for analysis:

Soil samples separated from each liverworts species brought to laboratory and spread out on an aluminum tray brown paper. Coarse, stones, gravels, rhizoid pieces, plant debris, and other residue are removed. Large lumps of moist soil are broken by hand. The air dried soil samples are crushed in mortar and pastels and then sieved through 2 mm nylon sieve.

Physical characteristics

Physical characteristics like electrical conductivity, pH, soil colour, texture, structure, soil constitution and new growths are tested as follows -

Soil colour:

Colour of the soil is one of the most outstanding morphological feature. The variation in colour of different horizons serves as one of the factors that leads to recognition of different soil types. Sometimes, colour of soil is mottled or variegated. Moist soil is usually more dark than when it is dry. Soil colour varies considerably. Some important soil colours are black, red, yellow brown, gray and white. Soil colour is due to colour of the predominant soil particles and weathering complex and both inorganic and organic contents that impart colour to the soil mass as like bellow -

a) Black colour due to the accumulation of humus or decaying organic matter. Also magnetite and oxides of manganese and titanium, if present in sufficient quantities, also

impart black colour. In arid regions, black alkali soil and its patches due to accumulation of sodium carbonates usually infertile.

b) Red colour of soil is mainly due to presence of iron oxide, in form of ferric oxide. Red soil are usually found in high temperature seems to be necessary for their development. In moist tropical regions laterite possess red and orange colour due to presence of various hydrated iron oxides.

c) Reddish brown soil due to hydration of turgite iron oxide and yellow brown, black by hydrated goethite iron oxides.

d) Gray colour soil is due to removal of bases like iron from the soil mass by leaching. It is formed in humid regions.

e) White colour soil usually indicates a sandy soil. A high contents of silica or $CaCo_3$ imparts a white colour to the soil.

Soil texture:

Texture denotes the size of individual soil particles. Particle of various size have distributed in different horizons, which form texture profile. This profile differ by relative amount of coarse and fine particles.

When small sized particles present in it, the soil may be termed as coarse or fine. If number of big particles is large like in a sandy soil termed as a coarse texture and number of soil particles are small as in clay soil, it is said to have a fine texture. Absorption, retention, and circulation of water determined by the size of particles.

Textural composition:

The various soil separates can be grouped into three main factors *viz.* sand, silt and clay. Sand represents as biggest, clay as the smallest, and silt as intermediate particles. Soil classified into textural Classes such as - sandy soil, clay soil and loamy soil. Silt is a very valuable constituent of soil. Clay particle plays a very important role in

soil fertility. Soil separates like clay (< 0.002 mm), silt (0.002 to 0.05 mm), and sand (0.05 to 2 mm) obtained by mechanical analysis.

The recent system elaborated into twenty textural groups are as - coarse sand, sand, fine sand, very fine sand, loamy coarse sand, loamy sand, loamy fine sand, loamy very fine sand, coarse sandy loam, sand loam, fine sandy loam, very fine sandy loam, loam, Silt loam, sandy clay loam, clay loam, silty clay loam, sandy clay, silty clay and clay.

I. Mechanical analysis:

The main aim is to determine the size of particles. It is usually done by crushing the soil lightly in a wooden mortar and then material is next passed through a 2 mm sieve to separate stones and gravel.

II. By sieve method:

Stones and gravels removed from soil by passing through sieve mesh and small particles like silt and clay separated.

Soil structure:

Cementing materials taking part in aggregate formation are colloidal clay, iron and aluminum hydroxides. In the system of classification of structure, based on nature of aggregates, as revealed by the examination of soil granules under microscope.

According to Russian system, the soil structure classification is based on nomenclature and approximately size (mm) as Cubic granular (< 5 mm), Nutty (5- 20 mm), Prismatic (10-50), Columnar (30-50), Platy (3-5 mm), Foliated (< 1).

Soil constitution:

It indicates the degree of compactness or looseness to which soil material is built, during the process of soil development. Compactness by the infiltration and deposition of

cementing materials like silica, lime, iron and aluminum hydroxides etc. In case of constitution compactness, it offers to resistance to penetration of foreign material. On the other hand, the constitution may be loose and porous, then activity in vice-versa.

New growth:

Certain compounds through physicochemical reactions accumulate in the soil mass like lime nodules, in chernozem and black cotton soil. Gypsum occurs at various depths in many soil types. The new formation occurs in form of streaks and patches (Kamat, 1959).

Other physical characteristics: Soil reaction (pH) and Electrical conductivity (E.C.):

The pH measured by pH meter and electric conductivity by Electric conductometer.

The negative log of activity of H^+ ion in soil solution indicates soil pH. When activity of H^+ ion in the soil solution increases then soil pH decreases, indicating an increase of acidity level. Soil pH affects on rhizoid growth, plant nutrition, yield production, microbial activity and other soil processes by influence.

Chemical characteristics:

Soil mineral matter like gravel (> 2 mm), sand (2 mm > 0.2 mm), silt (0.02-0.002) contains particles of rock and minerals while clay (0.002) consist of weathered products of rocks. Small quantities of oxides of iron, aluminum, titanium, manganese, carbonates of calcium and sulphates. Silt and clay contains primary minerals of mica, feldspar and hematite. Clay is the highly active portion of soil and it has high water holding capacity.

The element analysis was made at Soil Testing Laboratory, Someshwarnagar; Krushi Vidnyan Kendra, Shardanagar and Post-Graduate Research Centre, Department of Botany, Tuljaram Chaturchand College, Baramati (Dist. Pune). Available nitrogen (N)

are estimated by method of Subbaiah and Asija, (1959); Phosphorus (P) by Olsen *et al.*, (1954); Organic carbon percentage (C%) by Walkley-Black, (1934) and Potassium (K) by Jackson, (1958).

D. Biological characteristics (Microbiology) :

Soil is the habitat of a large number of micro flora. The recent discovery of antibiotic activity by soil micro flora open up new possibilities of investigation. Soil contains algae, and microorganisms such fungi, protozoa, actinomycetes, bacteria. The various organisms that inhabit both macro and microelements in soil.

Microbial association :

Activities of some microorganisms are beneficial to each other. Like all living organisms, microbes require energy for their metabolic processes. The supply of energy is therefore, one of the chief factors in determining the biological activity in soil.

Fungi depends on organic combined carbon which obtain from carbohydrates like sugars, starch, hemicelluloses, cellulose etc. Some fungi utilize fats and fatty acids as source of carbon. Fungi obtain their other nutrients from soluble mineral matter. Environmental factors influencing and determining the bacterial population and it fluctuates considerably from season to season.

Liverworts associated soil samples are categorized as per altitudinal areas and examined. The fungal isolation by 'serial dilution method,' and identifications was made by using standard literature (Alexopoulos *et al.*, 2002). The living organisms require energy from available sources such as carbon, nitrogen, sulphur, phosphorus, sodium, potassium, calcium, magnesium, iron, zinc, manganese, copper, cobalt, molybdenum and vitamins are some of important nutrients.

Culture medium for cultivation of fungal organisms under laboratory condition made. Preparation of media involves following important steps :

1. Prescribed amount of ingredients dissolved in an appropriate volume of distilled water.
2. Adjustment of the culture solution to a suitable pH.
3. Distribution of media into suitable containers.
4. Sterilization of the media by an appropriate method. Media and glass wares may be sterilized by physical methods that means, by dry heat (infra red radiation), and radiation (ultraviolet radiation). Also by chemical methods using disinfectants.

Preparation of culture media:

i. Potato Dextrose Agar (PDA) :

Composition : Peeled potato (200 g)
Glucose (15 g)
Agar-agar (20 g)
Distilled water 1 liter to make the final volume, at 7 pH.

Procedure : Pilled potato's are made into thin slices crush, and filtered through muslin cloth. Then, in the extract weighted quality of glucose solution and volume made up to 1000 ml. Medium is sterilized in autoclave at 15 lbs pressure for 20 minutes, while keeping the medium plugged it into 250 ml conical flask and this media used for isolation of organisms.

Agar-agar : It has m. p. approximately 92 ^{0}C while solidification point is near about 42 ^{0}C. Due to these peculiarity, there is wide range between its melting point and solidification point. It also supplements additional growth factor to the basic medium.

ii. Nutrient Agar Media (NA) :

Beef extract	- 6 g
NaCl	- 5.0 g
Peptone	- 10 g
Agar	-15 g
Distilled water	- 1 liter
Adjust pH to 7.0 to 7.5	

Methods of sterilization: Various methods of sterilization are followed -

1) Chemicals - Chemicals are used as cleaning solutions like $K_2Cr_2O_7 + H_2SO_4$, mercuric chloride, alcohol, formalin etc.
2) Light - Ultra Violet light rays are used for surface sterilization.
3) Autoclave - It is general method of sterilization all media soils, pots etc. material can be sterilized at 15 lbs pressure for 20 minutes. The organisms are killed, and media or glassware's whatever it may be sterilized.
4) Dry heat (Hot air oven) - This method is useful for sterilization of glass wares which used for this preparation. Material or glass wares first clean, and then placed at 100 ^{0}C for 4 hrs.

Isolation of pure culture:

Isolation of microorganisms from soil and individual organisms can multiplies to form distinct separate colonies on media.

Plating:

Soil samples (1 g) are taken and added into 100 ml distilled water. After shaking well, dilutions were made serially up to 10^{-6} Then, each dilution spreading on media Petri dish, incubated at 30 ^{0}C for 24-48 h for fungal, and 37^{0}C for bacterial isolation. Finally, colonies observations are made by routine standard methods.

E. Antimicrobial screening of thalloid liverworts:

Extraction of the following identified thalloid liverworts species was made with different organic solvents, and aqueous:

1. *Fossombronia indica* St.
2. *Sewardiella tuberifera* Kash.
3. *Exormotheca tuberifera* Kash.
4. *Asterella angusta* St.
5. *Plagiochasma articulatum* Kash.
6. *P. appendiculatum* L.
7. *P. simulensis* Kash.
8. *Targionia hypophylla* (Mich.) L.
9. *Cyathodium tuberosum* Kash.
10. *Riccia discolar* L.
11. *R. fluitans* L.

Test microorganisms and their maintenance:

In all eight microbial strains are tested for their response to thalloid liverworts extracts. They are obtained from National Collection of Industrial Microorganisms (NICM), Biochemical Sciences Division, National Chemical Laboratory, Pune, India. These included four bacterial strains *viz. Bacillus substilis* (NCIM 2697), *Escherichia coli* (NCIM 2067), *Pseudomonas aeruginosa* (NCIM 2200), *Staphylococcus aureus* (NCIM 2492) and four fungal strains *viz. Aspergillus niger* (NCIM 507), *Fusarium moniliformae* (NCIM 1276), *Fusarium oxysporum* (NCIM 1072), and *Rhizopus stolonifer* (NCIM 1139). Prior to use tested organisms, they are sub cultured on nutrient agar (NA), and Saboraud's dextrose agar (SDA), Hi-media to ensure their viability and adequate density.

Chemicals: Organic solvents like acetone, ethanol, and petroleum ether, Nutrient agar media (NA), Muller-Hinton agar media (MHA, Fluka), and Saboraud's dextrose agar (SDA) media are used.

Sterilized distilled water and antibiotics amphicillin and nystatin.

Other Requirements: Dry nutrient agar plates, cork borer, paper discs, pipettes and petri dishes (sterilized), spreader and forceps etc.

Agar media preparation:

a) Nutrient agar media (NA) preparation:

Composition -

Beef extract	- 6 g
NaCl	- 5.0 g
Peptone	- 10 g
Agar	-15 g
Distilled water	- 1 liter
Adjust pH to 7.0 to 7.5	

Nutrient agar (NA) media is prepared, sterilized and poured into sterilized Petri dishes, each containing 20 ml. After plates have solidified, a quadrant is marked on the lower surface of the Petri dishes then, 0.2 ml suspension of organism spread on all over the plates with a glass spreader.

b) Muller-Hinton agar media (MHA) preparation:

Composition -

Beef	- 3 g
Acid hydrolysate of casein	- 17.5 g
Starch	- 1.5 g
Agar	- 17 g
pH	- 7.2 to 7.4

c) Saboraud's dextrose agar (SDA) media preparation:

Composition -

Mycological Peptone	- 10 g
Maltose/Dextrose	- 40 g
Agar	- 15 g
Distilled water	- 1 liter
pH	- 6.8 to 7.0

Extraction :

Thalloid liverworts fresh plant material are treated with Tween-20 to remove epiphytic hosts which found on the surface, extensively washed in tap and distilled water, then dried on filter paper. Afterwards extraction made within four days. Plant material (10 g) was dried in open air at room temperature and extracted with acetone, ethanol, petroleum ether and distilled water, separately. Sensitivity of organisms to extracts was determined (Subramoniam *et al.*, 1998). The extracts shaking on rotary shaker (250- 300 r.p.m.) for 24 hrs and volumes of fractions are adjusted to 2 ml/g weight of plant material, then filtered it through cellulose acetate membrane paper (0.45 micron). Finally, filtrates were used to screen antibacterial and antifungal activities by applying extracts saturated discs.

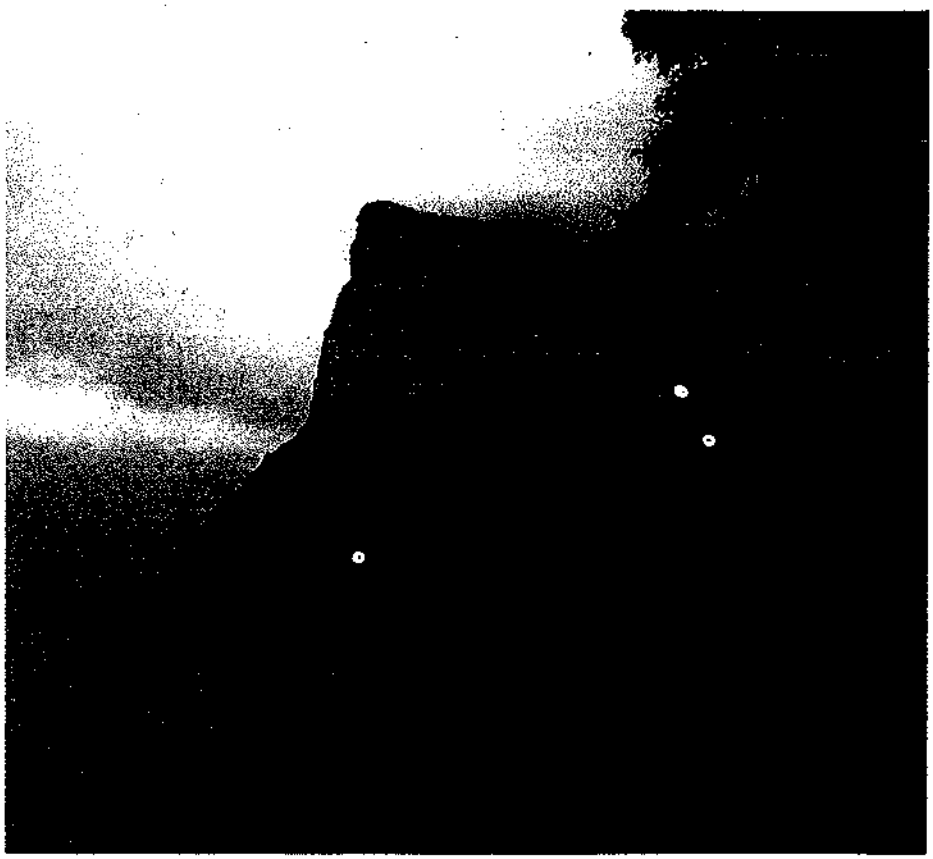
Agar diffusion assay and determination of antimicrobial activity :

Test organisms spread on agar plates and different bioactive extracts and antibiotics of high concentration (100, 50 μg^{-ml}) are applied in ways (disc diffusion) at the center of marked quadrate. After incubation, zone of inhibitions around the discs is measured. The diameter of zone inhibition is directly proportional to strength of bioactive extracts or antibiotics as well as sensitivity of microbes.

Extracts are screened for antibacterial and antifungal activities through 'disc diffusion assay method,' using 100 µl of suspension of tested microorganisms according to National Committee for Clinical Laboratory Standards (NCCLS, 1999, 2000; Santra *et al.*, 1999; Veljic *et al.*, 2009). This suspension contained 2 $x10^8$ CFU/ml for bacteria and 2 $x10^6$ CFU/ml spores for fungal strains. Muller-Hinton agar (MHA, Fluka) and Saboraud's dextrose agar (SDA), sterilized in a flask and cooled to 45-50 °C, are distributed in sterilized Petri dishes.

In antimicrobial screening, Whatmann no.1 filter paper discs (6 mm) Schleicher are individually impregnated with 10 µl of extracts, and then placed on to the agar plates, which had been previously inoculated with tested microorganism. Petri plates was kept at 4 °C for 2 h. The plates inoculated with bacteria, are incubated at 37 °C for 24 h and with fungal strains at 30 °C for 48 h. The diameter zone of inhibitions are measured in millimeters and mean values of inhibitory zones are accounted for results. The antibiotics amphicilin and nystatin served as a positive control.

4. CHARACTERIZATION OF SELECTED THALLOID LIVERWORTS EXTRACTS.



Characterization of selected thalloid liverworts extracts.

A. *Asterella angusta* St.

B. *Plagiochasma simulensis* Kash.

C. *Targionia hypophylla* (Mich.) L.

D. *Cyathodium tuberosum* Kash.

   a. Susceptibility of organisms.

   b. Effect of pH.

   c. Effect of temperature.

   d. Effect of detergents.

   e. Effect of enzymes.

   f. Solubility of bioactive components.

   g. Determination of shelf life.

   h. Thin Layer Chromatography (TLC).

   i. Determination of MIC and MFC value's.

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g. Determination of shelf life.

h. Thin Layer Chromatography (TLC).

i. Determination of MIC and MFC value's.

Chapter 4. : Characterization of selected thalloid liverworts extracts.

4. F) Characterization of selected thalloid liverworts extracts:

The present was undertaken with a view to characterize the antimicrobial properties of selected thalloid liverworts from Western Ghats, Maharashtra. The extracts have been subjected to characterize the antimicrobial properties by sensitivity of strains, by effect of pH, temperature, detergents, enzymes, shelf life, Rf values and minimum inhibitory concentration (MIC) values determination.

a. Susceptibility of target organisms to fresh and stored extracts of selected thalloids :

In antimicrobial screening, Whatmann No.1 filter paper discs (6 mm) are individually impregnated with 10 µl of stored and fresh extracts separately and then placed on to the agar plates, previously inoculated with tested microorganism. The Petri plates are kept at 4°C for 2 h. The plates inoculated with bacteria, are incubated at 37 °C for 24 h and with fungal strains at 30°C for 48 h. The diameter of zone inhibitions are measured in millimeters and the mean values of inhibitory zone were accounted for results (NCCLS, 2000; Mansour, 2005). The antibiotics, amphicillin and nystatin served as a positive control.

b. Effect of pH on activity and stability of bioactive components from selected thalloid liverworts extracts :

To determine the effect of pH on stability of bioactive components. 100 µl of bioactive extract was mixed with 1 ml of o.1 N phosphate buffer of varied pH in various tubes, incubated for one hour at 30 °C and residual antifungal activity in each tube was determined against target organisms such as *A. niger*, *F. moniliformae*, *F. oxysporum*, and *R. stolonifer*.

c. Thermal stability of antibiotic components of selected thalloid liverworts extracts:

To determine the effect of temperature on stability of bioactive components. Screw capped ampoules, each with 100 µl of bioactive extracts are kept at temperatures 30°C, 40°C, 50°C, and 60°C for one hour in water bath. The bioactive extracts cooled to room temperatures, and volumes are brought to the original and the residual antifungal activity was determined against target organisms (Mansour, 2005).

d. Effect of detergents on antibiotic activity of selected thalloid liverworts extracts:

Susceptibility of bioactive compounds to denaturation by various detergents *viz.* Tween 20, and Cetrimide was determined by mixing detergents with bioactive extracts, incubating them at 30°C for 6 hrs. Detergents was dissolved in distilled water at concentration of 0.01 g/ml. 100 µl of bioactive extract was mixed with 100 µl of detergent and incubated at 30°C (Munimbazi and Bullerman, 1998). Detergents added to distilled water are used as controls to check effect of detergents themselves on target organisms.

e. Effect of enzymes on antibiotic activity of selected thalloid liverworts extracts:

The sensitivity of bioactive extracts to denaturation by enzymes Protease K and Lipase was tested. Both enzymes are obtained from Sigma Chemicals Co. and dissolved in distilled water at conc. 1 mg/ml. 100 µl of bioactive extract solution was mixed with 100 µl of enzyme and incubated at 30 °C for 3 hrs. The bioactive extracts without any enzyme, served as control (Augustine, 2004). The residual antifungal activity of the mixture was tested against target organisms by 'agar disc diffusion assay' method.

f. Solubility of bioactive components of selected thalloid liverworts in different organic solvents and distilled water extracts:

Dry powder extracts of thalloid liverworts and their extraction was made with different organic solvents like acetone, ethanol, petroleum ether and distilled water, separately. Then solubility is observed.

g. Determination of shelf life of bioactive components from selected thalloid liverworts extracts:

The effect of storage time on antifungal activity of bioactive component's (100 µl) was determined by storing ampoules at 4°C for time 1 week, 1 month, 2 month and 3 months. After specified storage period, 100 µl extracts from each ampoules was added in well prepared Sabouard's dextrose agar plates already, inoculated with the target organisms i.e. *Aspergillus niger*, *Fusarium moniliformae*, *F. oxysporum* and *Rhizopus stolonifer*. The results were recorded after incubation at 30°C for 4 days.

h. Chromatography of selected thalloid liverworts extracts:

Crude extracts of some thalloid liverworts are analyzed for Thin layer Chromatography (TLC), was carried out using precoated glass TLC plates: normal phase (silica, Merk) (Ludwiczuk and Asakawa, 2010). Rf values determined by TLC, in Post-Graduate Chemistry department at Vidya Pratishthan's Arts, Science and Commerce College, Baramati.

Separation and isolation:

Crude acetone extracts of selected thalloids eluted with ethyl acetate: hexane (1:1) and pure hexane, step gradient fractions. The most active hexane fractions was subjected to chemical analysis to determine the classes of compounds present in it (Wagner *et al.*, 1984). The presence of alkaloids (Dragendroff's reagent and Mayer's reagent), coumarines (Borntagrs reaction), flavonoids (Shinoda test), steroids (Lieberman

Burchard test), steroid and terpenes (Vanillin- sulphuric acid reagent) are analyzed. The fraction was subjected to silica gel Thin Layer Chromatography (TLC) using chloroform as a solvent. The chromatograms are sprayed with various reagents to detect the presence of various classes of compounds by exposing to UV fluroscence (Subhisha and Subramoniam, 2005).

i. Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) value's of bioactive component from selected thalloid liverwort extracts against different target organisms:

Minimum inhibitory Concentration (MIC) :

Minimum inhibitory concentration (MIC) of an extract and antibiotic is that concentration which just inhabits the growth of a suitable primarily depends on the age of organism concerned.

The MIC and MFC value's of bioactive compounds were determined by 'broth tube dilution' procedure, using two fold dilution in Saboraud's dextrose broth at 28 °C temperature (Santra *et al.*, 1999; Cappuccino and Sherman, 1999). Antimicrobial substances may have cidal or static type of activity. Cidal agents have a capability of inhabiting growth of microorganisms, and have MFC values that are close to MIC values. MFC values of bioactive compounds are determined by sub culturing 50µl from tube, not visibly turbid and spot inoculating on to Saboraud's dextrose agar plates.

Requirements:

Broth cultures (24 h) : Broth cultures of test fungi *viz. A. niger* (NCIM 507),
F. moniliformae (NCIM 1276),
F. oxysporum (NCIM 1072) and
R. stolonifer (NCIM 1139).

Antibiotics : Nystatin and sterilized distilled water.

Other requirements : Culture tubes, Pipettes etc.

Nutrient broth preparation: Beef extract - 6 g
Peptone - 10 g
Distilled water - 1 liter

Nutrient broth was dispended in tubes, each containing 5 ml of medium. The tubes are plugged and autoclaved. *i.e.* 100 ml in 20 tubes.

I. a) Preparation of thalloid liverworts extracts :

Extracts were prepared with ethanol and distilled water.

b) Preparation of antibiotic solution :

The sterilized distilled water (10 ml) and antibiotic mixed in vortex mixer, from which 2 ml of this solution is taken by a sterilized pipette and then mixed with 98 ml of sterilized distilled water in a flask so that, the concentration of nystatin, become 20 µg/ml. The whole thing is done aseptically.

II. Addition of extracts or antibiotic in media:

The tubes containing media of 5 ml each are arranged in a test tube rack. They are labeled with 0 µg, 1 µg, 2 µg, ...10 µg each of which designates the amount of extract or antibiotic in that tube containing 5 ml. Since, the original organic solvent extracts concentration is (2 g in 20 ml), from which required concentration extracts is attained by addition of solvents in the following ways:

Substrate (ml)	Concentration of extracts in media (µg/ml)										
	0	1	2	3	4	5	6	7	8	9	10 µg
Medium	5	5	5	5	5	5	5	5	5	5	5
Solvent	5	4.5	4	3.5	3	2.5	2	1.5	1	0.5	0
Extracts	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Total volume	10	10	10	10	10	10	10	10	10	10	10

The original antibiotic solution is 20 µg/ml concentration, the required concentration of antibiotic is attained by addition of water same as above, in the following way -

Substrate (ml)	Concentration of antibiotics in media (µg/ml)										
	0	1	2	3	4	5	6	7	8	9	10 µg
Medium	5	5	5	5	5	5	5	5	5	5	5
Solvent	5	4.5	4	3.5	3	2.5	2	1.5	1	0.5	0
Antibiotic	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Total volume	10	10	10	10	10	10	10	10	10	10	10

III. Inoculation :

In each tube, broth culture (0.1 ml) is added. Inoculation of one set of tubes containing a particular extracts and antibiotic with a particular organism is previously determined. After addition of microorganisms suspension or culture, it is mixed thoroughly in a vortex mixture. For bacterial culture use, it is incubated at 37°C for 24 hrs

and for fungal culture, at 27-30°C for 24 hrs After proper incubation, the tubes are checked to find out, in which tube growth has occurred and in which it has not. The concentration of extracts or antibiotic in which growth has just stopped gives the MIC of the extract or antibiotic for that organisms (Santra *et al*., 1999).

5. RESULTS AND DISCUSSION

Results and discussion

A. Surveying.

B. Collection and Identification of thalloid liverworts.

C. Physico-chemical characteristics of associated soil.

D. Biological characteristics of associated soil.

E. Antimicrobial screening of thalloid liverworts.

F. Characterization of selected thalloid liverworts extracts.

   a. Susceptibility of organisms.

   b. Effect of pH.

   c. Effect of temperature.

   d. Effect of detergents.

   e. Effect of enzymes.

   f. Solubility of bioactive components.

   g. Determination of shelf life.

   h. Thin Layer Chromatography (TLC).

   i. Determination of MIC and MFC value's.

Chapter 5. Results and Discussion

A. Surveying: MAP I-IV

B. Identification of thalloid liverworts : (Table 5-A, 1)

Identification of thalloid liverworts was performed using previous bryophytic taxonomy literature (Schuster, 1958c, 1979). Identified specimens were then verified through comparison with material preserved in herbarium voucher specimens at department of Botany, Tuljaram Chaturchand College, Baramati. At least 13 species of thalloid liverworts belonging to 8 genera, distributed over 5 families, from which 11 species used for physicochemical, biological and antimicrobial screening.

The identified thalloid liverworts from different altitudinal areas of localities are as -

Locality : Rajgad

1. *Asterella angusta* St.
2. *A. reticulate* Kash.
3. *Plagiochasma articulatum* Kash.
4. *P. appendiculatum* L.
5. *P. simulensis* Kash.
6. *Targionia hypophylla* (Mich.) L.
7. *Cyathodium tuberosum* Kash.
8. *Riccia discolor* L.
9. *R. fluitans* L.

Locality : Purandar

1. *Fossombronia indica* St.
2. *Sewardiella tuberifera* Kash.
3. *Exormotheca tuberifera* Kash.
4. *Asterella angusta* St.

5. *Plagiochasma articulatum* Kash.
6. *P. simulensis* Kash.
7. *Targionia hypophylla* (Mich.) L.
8. *Cyathodium tuberosum* Kash.
9. *Riccia melanospora* Kash.
10. *R. discolor* L.

Locality : Sinhagad

1. *Sewardiella tuberifera* Kash.
2. *Exormotheca tuberifera* Kash.
3. *Plagiochasma articulatum* Kash.
4. *P. simulensis* Kash.
5. *P. articulatum* Kash.

Locality : Kas;Satara

1. *Fossombronia indica* St.
2. *Plagiochasma appendiculatum* L.
3. *P. simulensis* Kash.
4. *Targionia hypophylla* (Mich.) L.
5. *Cyathodium tuberosum* Kash.
6. *Riccia fluitans* L.
7. *R. discolor* L.

Locality : Lonawala and Khandala

1. *Exormotheca tuberifera* Kash.
2. *Asterella angusta* St.
3. *Plagiochasma simulensis* Kash.
4. *Cyathodium tuberosum* Kash.
5. *Riccia discolor* L.

Locality : Panhala

1. *Plagiochasma articulatum* Kash.
2. *P. simulensis* Kash.
3. *Cyathodium tuberosum* Kash.

Locality : Mahabaleshwar

1. *Fossombronia indica* St.
2. *Sewardiella tuberifera* Kash.
3. *Plagiochasma articulatum* Kash.
4. *P. simulensis* Kash.
5. *Targionia hypophylla* (Mich.) L.
6. *Cyathodium tuberosum* Kash.

Locality : Raigad

1. *Asterella angusta* St.
2. *Plagiochasma articulatum* Kash.
3. *P. simulensis* Kash.
4. *Cyathodium tuberosum* Kash.

Locality : Rohideshwar

1. *Asterella angusta* St.
2. *Plagiochasma articulatum* Kash.
3. *P. simulensis* Kash.
4. *Sewardiella tuberifera* Kash.
5. *Targionia hypophylla* (Mich.) L.
6. *Cyathodium tuberosum* Kash.

The 11 species of thalloid liverworts, belonging to 8 genera, distributed over 5 families are identified from different altitudinal areas of localities are as arranged as per system of classification by Schuster (1958c, 1979).

5 - A, **Table No. 1:** A total number of thalloid liverworts from each locality of Western Ghats, Maharashtra.

Sr. No.	Name of the species	A	B	C	D	E	F	G	H	I	J	K	L	M
1	*Fossombronia indica* St.	–	+	–	–	+	–	–	–	+	–	–	–	–
2	*Sewardiella tuberifera* Kash.	–	+	+	+	–	–	–	–	+	+	–	–	
3	*Exormotheca tuberifera* Kash.	–	+	+	–	–	+	+	–	–	–	–	+	–
4	*Asterella angusta* St.	+	+	–	–	–	+	+	–	–	–	+	–	–
5	*Plagiochasma articulatum* Kash.	+	+	+	+	–	+	+	+	+	+	+	–	–
6	*P.appendiculatum* L.	+	–	–	–	+	+	+	–	–	–	–	–	–
7	*P. simulensis* Kash.	+	+	+	+	+	+	+	+	+	+	+	+	+
8	*Targionia hypophylla* (Mich.) L.	+	+	+	+	+	+	+	–	+	+	–	–	+
9	*Cyathodium tuberosum* Kash.	+	+	+	+	+	+	+	+	+	+	+	+	+
10	*Riccia discolar* L	+	+	–	–	+	+	+	–	–	–	–	–	–
11	*R. fluitans* L.	+	–	–	–	+	+	–	–	–	–	–	–	–
	Total	**8**	**9**	**6**	**5**	**7**	**9**	**8**	**3**	**6**	**5**	**4**	**3**	**3**

Present (+), Absent (-)

A-Rajgad, B- Purandar, C- Sinhagad, D- Rayereshwar, E- Kas;Satara, F- Lonawala, G-Khandala, H-Panhala, I-Mahabaleshwar, J-Pachgani, K-Raigad, L-Shivthargad, M-Rohideshwar

PLATE - I

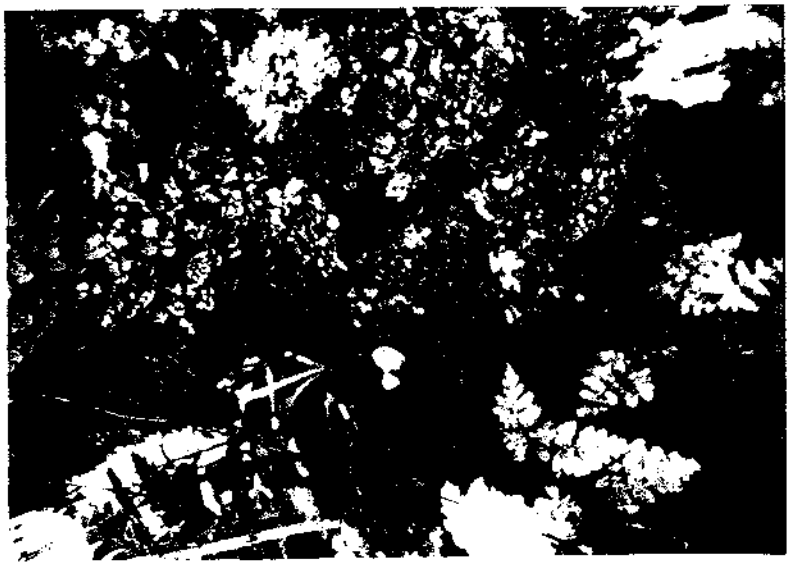
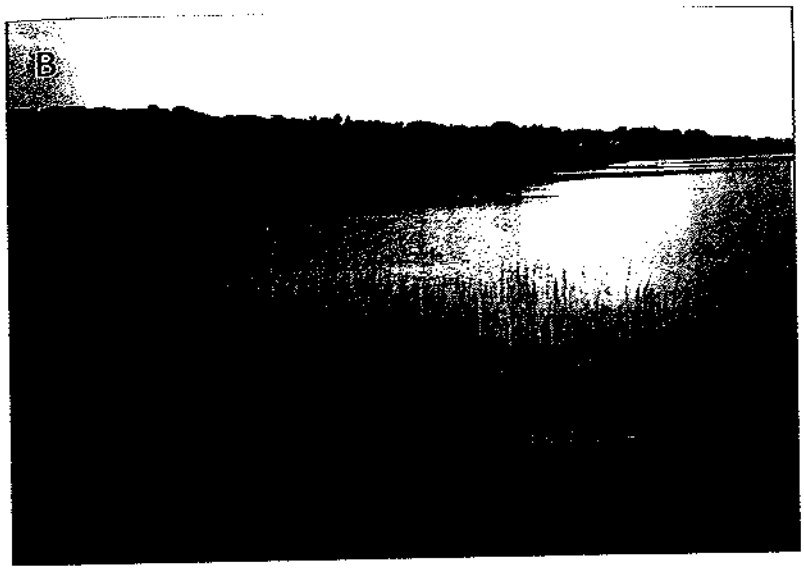


Photo Plates of habitats: A. Rajgad; B. Purandar; C. Sinha**gad.**

PLATE - II



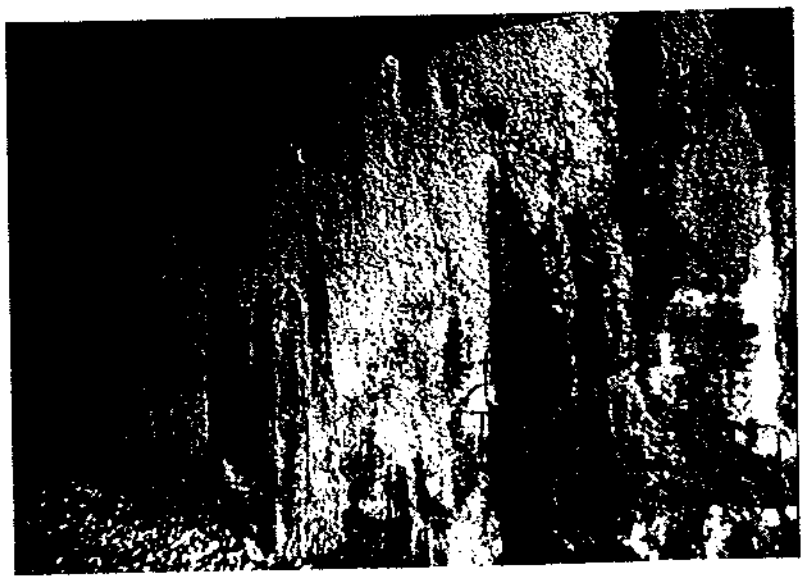


Photo Plates of habitats: A. Rayreshwar; B. Kas-Satara; C. **Lonawala.**

PLATE - III

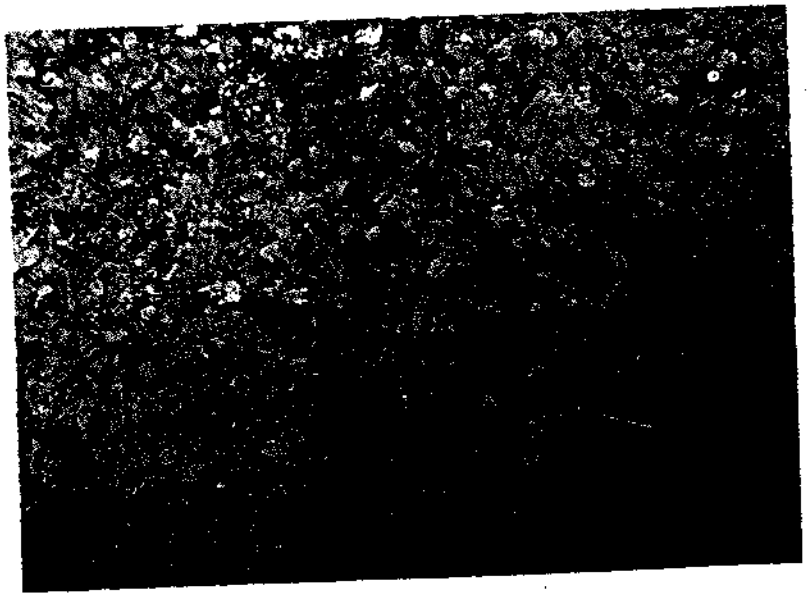
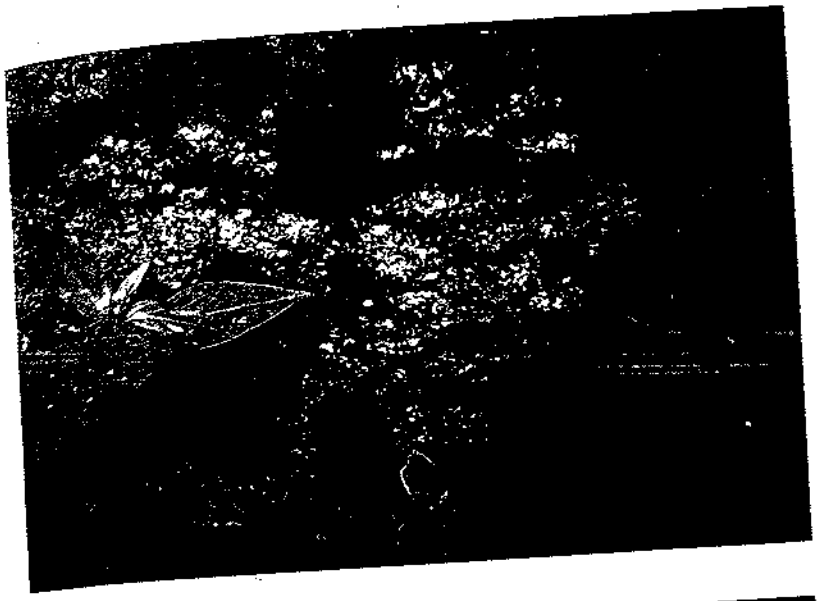


Photo Plates of habitats: A. Khandala; B. Panhala; C. Mahabaleshwar.



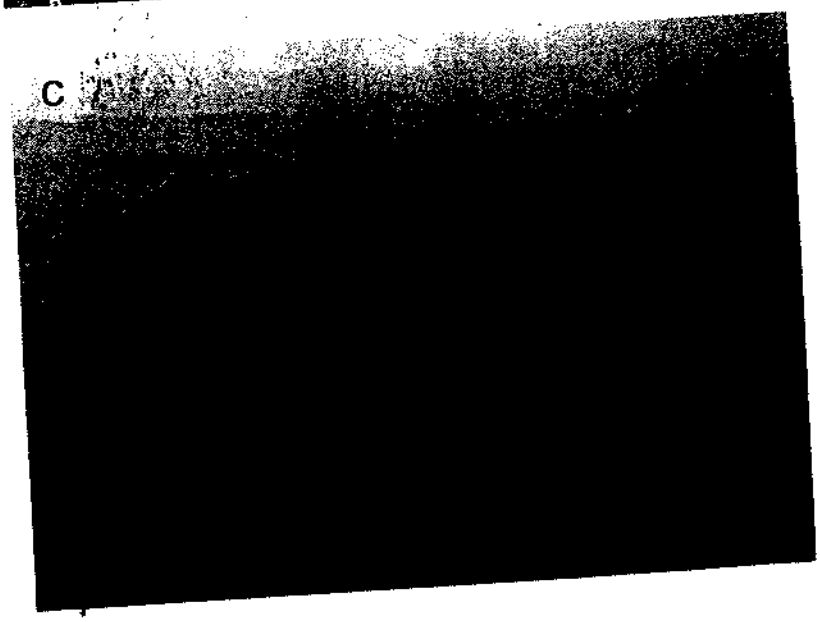


Photo Plates of habitats: A. Shivthargad ; B. Raigad ; C. Rohehideshwar.

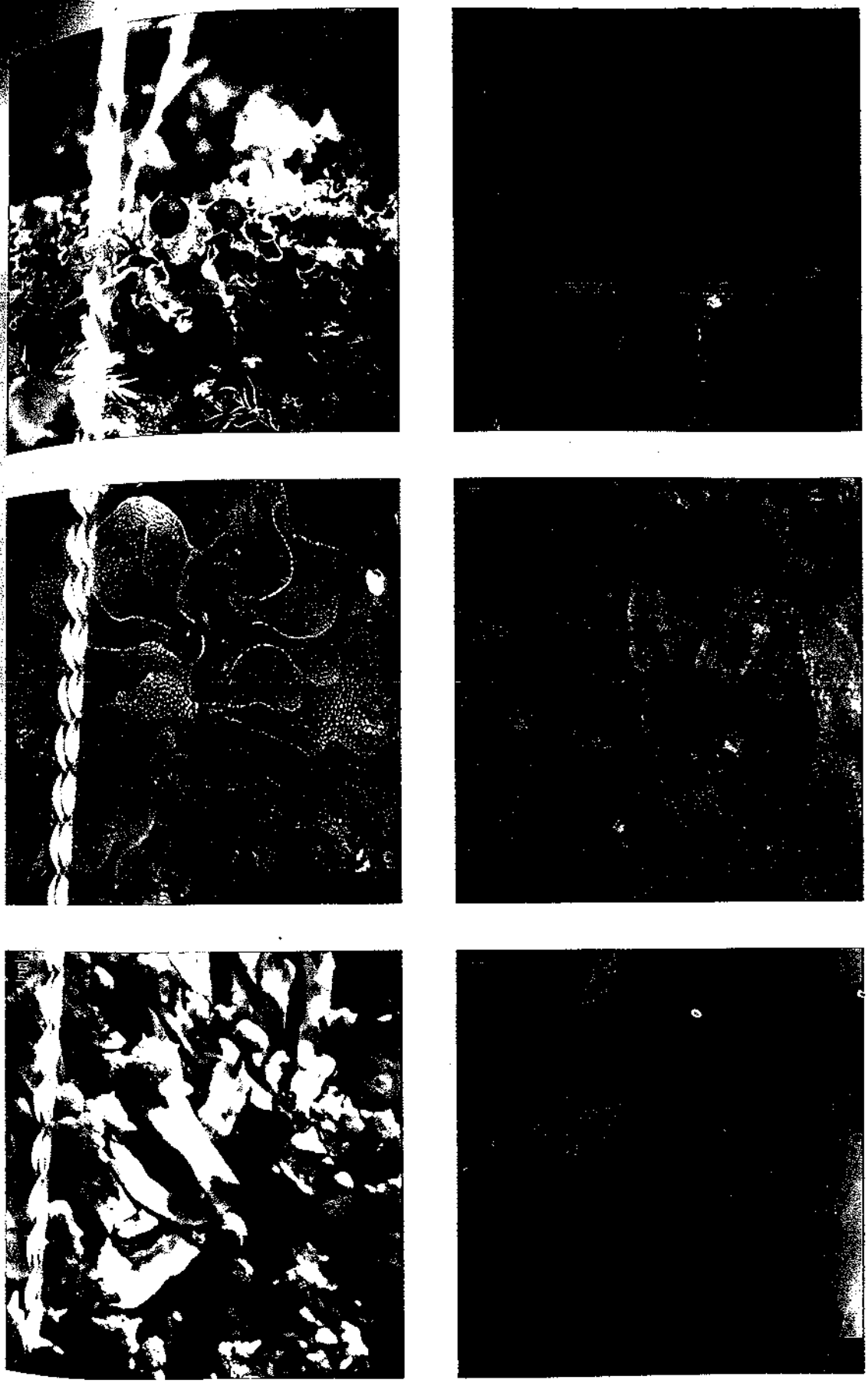
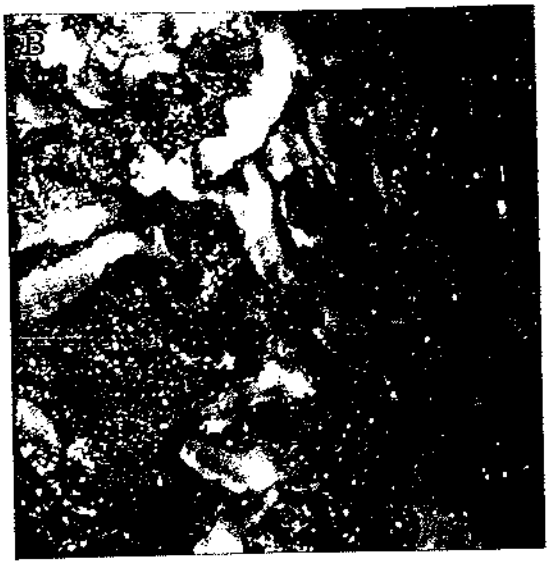


Photo Plates of thalliod liverworts: A. *Fossombronia indica* St.; B. C. *Sewardiella tuberifera* Kash.; D. *Exormotheca tuberifera* Kash.; E. *Asterella angusta* St.; F. *Plagiochasma articulatum* Kash.



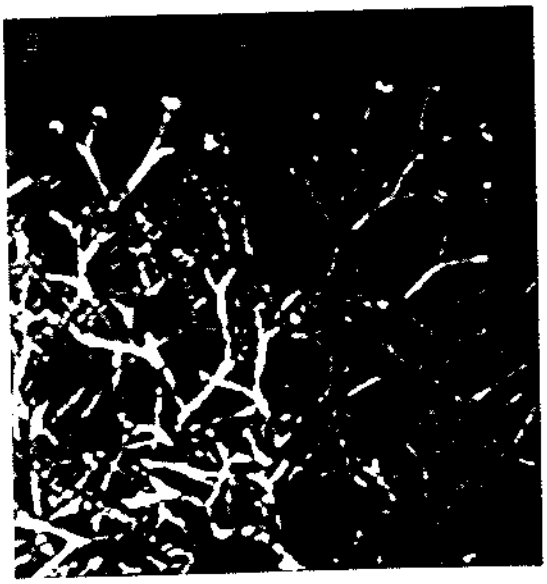


Photo Plates of thalliod liverworts: **A**. *Plagiochasma appendiculatum* **L.**; B. *P. simulensis* Kash.; C. *Targionia hypophylla* (Mich.) L.; D. *Cyathodium tuberosum* **Kash.**; E. *Riccia discolar* L.; F. *Riccia fluitans* **L.**

Class - Hepaticae

Order - Jungermanniales

Sub order - Metzgerineae

Family - Fossombroniaceae

(a) Genus - *Fossombronia* Raddi.

1. *F. indica* St.

(b) Genus - *Sewardiella* Kash.

2. *S. tuberifera* Kash.

Order- Marchantiales

Family - Marchantiaceae

(c) Genus - *Exormotheca* Mitt.

3. *E. tuberifera* Kash.

Family - Rebouliaceae

(d) Genus - *Asterella* Beauv.

4. *A. angusta* St.

(e) Genus - *Plagiochasma* L.

5. *P. articulatum* Kash.

6. *P. appendiculatum* L.

7. *P. simulensis* Kash.

Family - Targionaceae,

(f) Genus - *Targionia* (Mich) L.

8. *T. hypophylla* (Mich.) L.

(g) Genus - *Cyathodium* Kunze.

9. *C. tuberosum* Kash.

Family - Ricciaceae

(h) Genus - *Riccia* (Mich) L.

10. *R. discolar* L.

11. *R. fluitans* L.

Descriptive : Morphology and Taxonomy

KEY TO THE GENERA

1.	Plants thallose, with or without histological differentiation of leafy and then the archegonia don't stop the growth	2
1.	Plants leafy, leaves conduplicate or simple.	8
2.	Plants thallose with histological differentiation and sex organs on receptacles	3
2.	Thallose without histological differentiation or leafy, sex organs in groups, not stopping the growth of plants.	6
3.	Sex organs on the thallus, no receptacles.	*Riccia*
3.	Archegonia on receptacles.	4
4.	Receptacle sub-apical	5
4.	Receptacle terminal or dorsal	6
5.	Plants thin, pellucid.	*Cyathodium*
5.	Plants large, green, robust	*Targionia*
6.	Receptacle dorsal	*Plagiochasma*
6.	Receptacle terminal	6
7.	Receptacle with conical pseudoperianth	*Asterella*
7.	Receptacle without conical pseudoperianth	*Exormotheca*
8.	Plants lobes or leafy	*Fossombronia*
8.	Plants with distinct wing on either side	*Sewardiella*

A. Class - Hepaticae

Thalloid or foliose plants. When foliose, leaves are without midrib. Internal structure simple. Sex organs develop from a single initial cell. Sporophytes with foot, seta

and capsule, sporogenous tissue with spores and elaters (except, *Riccia*). Columella altogether absent.

Order - Jungermanniales

Plant body is thalloid or foliose. Foliose plant body is differentiated into a central axis and leaves. Leaves are always without midrib. Internally the gametophytes are little differentiated. Scales are absent. Rhizoids always smooth. Antheridia superficial, sometimes embedded in cavities, globular or club shaped with slender stalks. Archegonia generally in groups but, never raised on receptacles. Sporophytes with foot, seta and capsule. Capsules are multistratose and thick elaters are present. Dehiscence of the capsule usually by means of four valves.

Sub-order : Metzgerineae (Anacrogynae)

Gametophytes generally thallose but, sometimes foliose differentiating into stem and leaves. Sex organs on dorsal side. Archegonia in groups.

Family - Fossombroniaceae

Plants in the foliose forms, leaves in two rows, parallel to the stem or obliquely inserted, succubous, simple. Rhizoids always present. Male and female inflorescence scattered on the dorsal side or in groups. Archegonial cluster surrounded by an involucres. Sporophyte with foot, long seta and capsule. Capsule globose dehiscing or irregularly, to the base by four valves. Spore measures about 30 µ. Elaters long, 2-4 spiraled.

a) **Genus - *Fossombronia* Raddi.**

Fossombronia Reddi., in Atti Sce. Ital. Mod., **18** (1918).

Plants are mono or dioecious. Plants delicate, green to yellowish green, small to large, solitary or in patches. Stem simple, prostrate or procumbent, dichotomously

branched, dorsally flattened, with or without apical tubers. Leaves simple, quadrate to sub-quadrate, usually broader, narrow at the base succubous, obliquely inserted in two lateral rows, irregularly lobed. Antheridia orange-yellow, globose shortly stalked, aggregated towards apex at the base, young leaves on the dorsal surface of the stem. Archegonia pink in colour, dorsal on stem, usually solitary in proximity of leaf, sometimes mixed with antheridia toward the apex, bracts usually absent. Sporophyte differentiated into foot, seta and capsule. Capsule spherical or globose dark brown to blackish at maturity, dehiscence irregular, wall bistratose, spores usually large, tetrahedral, yellowish brown to dark-brown. Elaters small to large, branched or unbranched, pale yellow or yellowish brown spirals.

Fossombronia indica St. Sp. Hep. **Vol. VI**, p. 73 (1917). (Pl. V-A).

Plants are delicate, small and dioecious. Stem is 1to 7 mm long, prostrate, once or twice dichotomously branched. Leaves are simple broader than long, narrow at the base. They are obliquely placed on the stem, flat having entire or slightly wavy margin. Male plants are nearly of the same size as the female ones. The margins are slightly wavy, open on one side. Calyptra is delicate and thick at the base. Sporophyte is with a short seta and spherical capsule. The capsule is dark brown to blackish- brown. Spores are yellowish brown. Elaters are broad in the middle with acute to obtuse ends, yellowish brown spirals, less pigmented and loosely twisted.

Habitat : Shady places in association with mosses.

Locality : Kas;Satara, Panhala and Mahabaleshwar.

Date of Collection : September, 2010; November, 2010; August 2011.

Distribution in India : Manglore, South India (Stephani, 1917).

b) **Genus - *Sewardiella* Kash.**

Sewardiella Kash., New Phyt. Vol. XIV, p. 5 (1915).

Plants are dioecious, thallose, simple or forked, occurring in thick patches on rocks singly in shady places. Thallus showing attenuated wings, directed upwards. Dorsal

surface concave. Midrib thick, projecting ventrally with minute red scales in two rows. Wings multi-layered at the base, ascending margin wavy. Male and female plants are alike. Antheridia and archegonia are in cluster on the dorsal side of the midrib. Perianth bell-shaped. Calyptra thin, single layered. Sporangia one or more in each perianth. Capsule wall 1-3 layered. Spores reticulate. Elaters bi or trispiral.

Sewardiella tuberifera Kash. (Pl. V-B).

The thallus is light green in colour and looks like a fern prothallus. It possesses a thick midrib and delicate wings. The ventral surface bears numerous simple rhizoids and small scale towards apex. In the young plant the wings are ascending but, as it grows older they spread out somewhat horizontally. Sex organs begins to appear while the plant is very young.

Habitat	:	Plant may occur singly among mosses and grasses or may form large patches of overlapping individuals.
Locality	:	Purandar, Sinhagad and Mahabaleshwar.
Date of collection	:	October, 2009; August, 2011.
Distribution in India	:	Common at 5000 to 7000 ft., Mussoorie, Simla etc. (Kashyap., 1929).

Order - Marchantiales

The thalloid plants with air chambers on dorsal side, opening by pores. Rhizoids of two kinds; scales often present. Sex organs on dorsal side, arranged on receptacles in higher forms. Sporophyte with foot, seta and a capsule. Elaters often present.

Family- Marchantiaceae

Plants thalloid. Pores well defined. Sex organs in groups on the dorsal surface, often on long stalked receptacles. Sporogonium with a foot and a seta. Elaters well developed. Dehiscence by definite valves.

c) **Genus - *Exormotheca* Mitt.**

Exormotheca Mitt., in Godman Hist. Azores, P. 325 (1870).

Thallus dichotomously branched, lobes long, linear. Monoeceous or dioecious. Dorsal surface flat. Air chambers in one layer, full of simple assimilatory filaments. Stomata raised, simple. Midrib distinct ventrally. Scales large, densely imbricate, acuminate or blunt, with or without appendages. Antheridia usually along the mid-dorsal groove. Female receptacle stalked, arising from the apex. Spores areolate. Elaters yellowish, loosely bi-or-tri-spiral.

Exormotheca tuberifera Kash., New Phytol. **Vol. XIII**, p. 309 (1914). (Pl. V-D).

Thallus is twice or thrice dichotomously. Lobes are long, linear and firmly attached to the soil. Dorsal surface is green, often having a deep narrow groove with stomata. Pores variable in size. Assimilatory filaments are simple. Ventral surface is purple. Scales are hyaline or purple, overlapping. Antheridia are often in a groove behind the stalk of the female receptacle or along the midrib in the median groove in a zigzag. Female receptacle arise from a pit in the fork between two lobes. Stalk long with one rhizoidal groove. Capsule is fully exerted and is directed upwards like horns. Operculum with a few short elater-like cells suspended from it. Dehiscence of the capsule is by 4 irregular valves. Spores are tetrahedral, covered with conical papillae. Elaters are with spiral bands, occasionally branched.

Habitat	:	On the flat ground at moist shady places.
Locality	:	Purandar, Lonawala and Khandala.
Date of Collection	:	August, 2009 and September, 2010.
Distribution in India	:	Outer Himalaya; Garhwal, Mussoori, S. India.

Family- Rebouliaceae

Plants monoecious or dioecious. Pores simple, scales in two rows with appendages. Male receptacle sessile, cushion-like, horse-shoe-shaped. Female receptacle

terminal. Receptacle flat, concave or convex, four lobed. Capsule globose. Spores tetrahedral. Elaters simple, branched.

d) Genus - *Asterella* Beauv.

Plants are green thallose, terrestrial and prostrate. Plants monoecious or dioecious. Thalli are simple. Rarely divided, innovating from the apex and often with ventral (fertile) innovations. Dorsal layer low, chambers narrow, often very irregular, and with numerous secondary lamellae in one or several layers, empty or containing filaments. Ventral scales with appendages, are in one row on either side of the midrib. Male receptacles sessile, naked, disc shaped or cushion like, just behind the stalk of the receptacle on ordinary, main shoots or on small ventral shoots, papillae small. Female receptacles terminal on the main shoot, or on small ventral shoots, stalked. stalk with one rhizoidal furrow covered with scales, receptacles flat, convex conical or umbonate, usually 4-lobed, stomata barrel-shaped. Involucres arising from the margin of the lobes, thin, cup shaped or companulate. Archegonia are in each involucres. Sporangia one in each involucres. Capsule globose, shortly pedicillate, wall single-layered, lid of thick walled cells. Spores tetrahedral, reticulate-lamellate on the convex side, more or less yellow. Elaters short, simple or furcated mono or bi-spiral.

Asterella angusta St., Sp. Hep. **Vol. I** (Pl. V-E).

Plants are dioecious but, the male and female plants grow in mixed clones. The lobes are ovate, oblong, or linear oblong. They firmly adhere to the soil by a tuft of smooth or tubericulated rhizoids. The midrib is broad and conspicuous and margins slightly curl upwards. On the ventral surface of the thallus two rows of purple, one celled thin scales.

They are very conspicuous in the apical region protecting a growing part. They are triangular or lunate in shape. The dorsal surface of the thallus is marked by polygonal cell sand air pores of simple type. The air pores on the carpocephalum are barrel shaped.

Male receptacles are on the main thallus. Female receptacles are disciform with two or 4-6 involucres. Scales on carpocephalum are purple and linear. Perianth is horizontal. Some times directed upwards, ovate 2/3 exerted. Spores are tetrahedral, dark brown, closely reticulate reticulations often incomplete, margins papillate, papillae rounded or conical. Elaters unbranched, monospiral, and yellowish.

Habitat : On dry rocks, on walls near railway tracks.

Locality : Lonawala; Khandala, Mahabaleshwar, and Raigad.

Date of Collection : September, 2010; August, 2011.

(i) *Asterella angusta* St. Var. jamesii

A. angusta St. collected from Lonawala and Khandala near railway track. It was showing only an archegoniophore without formation of any sporophyte even after the rainy season is over. The plant with both type of rhizoids. On ventral surface 2 rows of scales present.

e) Genus - *Plagiochasma* L. et L.

Plagiochasma L. et L. in Lehm. Pug. **Pl. IV**, p. 13 (1832).

The plants monoecious or dioecious. Plants large, creeping in thick patches, and green. Scales in 2 rows with appendages. Male receptacle sessile when young, usually stalked at maturity. Receptacle more or less concave on dorsal surface, with barrel-shaped pores, 2-7 lobed, involucres large, inflated, bi-valved, margin of the valves involute in the young condition one of which opens at maturity while, the other remains folded inwards, each containing one archegonium and lather on one sporangium. Calyptra thin. Capsule short, with a large foot, opening by an indistinct lid. Elaters short bi-tri-spiral, sometimes uniformly thickened without spirals, yellowish.

Key to the species

1. Thallus distinctly articulated, female receptacle borne at the articulation .. *P. articulatum*

	Plants as a rule not articulated, female receptacle always dorsal	..	2
2.	Lobes broad, obcordate	..	*P. appendiculatum*
	Lobes narrow, linear	..	2
3.	Scale appendages not constricted ..	..	*P. simulensis*

(i) *Plagiochasma articulatum* Kash. New. Phytol. **Vol. XIII**, p. 320 (1914) (Pl. V-F).

Monoecious plant. Lobes are oblong-ovate flat with undulate margins and notched apex. Thallus about 40 mm long and 5-7 mm broad. Dorsal surface is dark green. Ventral surface is purple. Scales are purple, overlapping, each with 2 or 3 long, narrow purple appendages which bend over the growing point. Midrib is conspicuous, gradually passing into the lamina, ending in an acute margin. Receptacles are always terminal but, become dorsal at maturity due to vegetative growth. Male receptacles are horse shoe-shaped. Pores on the receptacles are simple is none or very short reaching up to 2 mm. Receptacles with 3- 4 lobes. Capsule wall is single layered. Spores are yellowish, reticulate, lamellate. Elaters are normally bispiral.

Habitat : On dry rockery.
Locality : Kas;Satara, Lonawala, Khandala, and Mahabaleshwar.
Date of Collection : September, 2010 and August 2011.
Distribution in India : Common at outer, Kumaon Himalaya (Kashyap., 1929), Nilgiris.

(ii) *Plagiochasma appendiculatum* L. Pug. **IV**, p. 14 (1832). (Pl.VI-A).

Plants are monoecious. Thalli form large thick patches. Lobes are oblong, obcordate. Each lobe measures 10- 40 mm in length and 4- 10 mm in breadth. Dorsal

surface is smooth and slightly concave or flat. Margins are undulate, areoles invisibles. Male receptacle are horse-shoe shaped, sometimes once or twice furcated surrounded by small scales. Pores on the receptacle are simple. Female receptacles are often with 5 to 9 lobes. Capsule wall is single layered. Spores yellowish reticulate-lamellate (including the wings), 2-3 reticulation in the diameter, wing is finely punctuate. Elaters are bispiral with uniformly thick walls with or without spirals.

Habitat	:	On rockery
Locality	:	Kas;Satara, Lonawala, Khandala, Mahabaleshwar and Raigad.
Date of Collection	:	September, 2010 and August 2011.
Distribution in India	:	Kumaon Himalayas up to 9000 feet; Simla, Mussoorie, Nainital, Nilgiriis.

(iii) *Plagiochasma simulensis* Kash. J. Bom. Nat. Hist. Soc. **Vol. XXV**, p.(1971). (Pl. VI-B).

Plants are monoecious or dioecious bluish green. They occure in dense patches and closely creeping. Plant body is dichotomously branched, 15 mm long and 4 mm broad. Each lobe is long, linear with rounded apex; margin is entire and purple. Dorsal surface is smooth and plane. Epidermal cells are thin walled. Pores are minute, simple. Ventral surface is purple. Scales are purple or hyaline, ovate to lanceolate, usually ending at the apex in a 2- celled filament. Male receptacles are mid- dorsal, either on different lobes of the thallus bearing female receptacles or on different plants, cushion-like, circular or notched anteriorly. Female receptacle is sessile or shortly stalked measuring 2 mm in length. Receptacles are concave dorsally. Spores are broadly reticulate, lamellate, 3-5 reticulations in the diameter (excluding the wing). Wing is 8 μ broad. Spores are 115 μ in diameter. Elaters long, closely 3-4 spiral, broad.

Habitat : On moist walls generally growing together with *P. articulatum*.

Locality : Kas;Satara, Lonawala, Khandala, Mahabaleshwar, and Raigad.

Date of Collection : September, 2010 and August 2011.

Distribution in India : Simla, S. India, Kodaikanal, Outer Himalaya.

Family - Targionaceae

Plants monoecious or dioecious. Air pores present or absent. Scales in two rows. Rhizoids smooth and tubericulated. Antheridia on dorsal or on main thallus in cushion, terminal or lateral, involucres terminal, ventral. Archegonia a few or many. Capsule globose, stalked. stalk small. Spores rounded, reticulate or muricate. Elaters are long, fusiform bi- or tri- spiral.

f) Genus - *Targionia* (Mich.) L. Sp. Pl. : 1136.

Plants thalloid, terrestrial and prostrate. Thallus simple or rarely dichotomously. Pores simple. Ventral scales into rows. Antheridia on the dorsal surface, short ventral innovations arising from the midrib or broad mid- dorsal cushions on the main shoots; ostioles papiliform. Involucres two, bi-valved, interlocked by means of small microscopic teeth on the cells of margins. Archegonia several, sporangium usually one in each involucres, rarely two. Perianth absent. Capsule shortly pedicillate, with a well developed foot, breaking through the calyptras, the two valves of the involucres separating, leaving a slit-like opening. Spores reticulate. Elaters long, bi-spiral.

Targionia hypophylla (Mich.) L. 1753 : Sp. Pl. : **1136**. (Pl. VI-C).

Plants are monoecious with dark green colour. Thallus is 10-12 mm long and 2-4 mm broad. Plants rarely grow dichotomously. Ventral side of the thallus with ventral

innovations and rhizoids. Apex is distinctly notched; dorsal surface is flat with distinct areoles. Ventral surface is purple coloured. Scales are triangular with mucilage papillae. The air chambers are with 2-3 celled partitions filled with distinct chloroplasts containing filaments of 2/4 cells of which some filaments are branched. Terminal cell of the filament is larger and with or without plastids. The assimilatory chambers are in one row only. The storage tissue is parenchymatous. Antheridia are found on small lateral innovations. Involucres is terminal and ventral with sub-globose keel having 5/6 archegonia but, only one (rarely two) archegonium develops into a sporophyte. Capsule is spherical with a single-layered wall, wall cells with thickened bands. Spores are reticulate with 4/5 reticulations across the diameter, with smaller reticulations arising from the centre of each large reticulation. Elaters long, are yellowish, simple or branched with bi-spiral bands.

Habitat	:	On moist ground or on exposed rocks.
Localities	:	Kas;Satara and Mahabaleshwar.
Date of Collection	:	September, 2010 and August 2011.

g) Genus - *Cyathodium* Kunze. 1854. Lehm. Pug. VI : 17.

Plants are monoecious or dioecious. Thalli thin, small, fan shaped, grouped in rocks or on grounds consisting of dorsal and ventral layer of cells separated by air spaces, divided by thin vertical portions. Thalli are dichotomously divided. Air chambers are in one layer, empty, with or without simple pores. Pores are large, bounded by concentric rings of cells. Scales minute, in two rows or totally absent. Rhizoids smooth, sometimes thick walled but, never tubericulated. Antheridia plenty, ostioles papillose. Capsule globose; seta small, slender delicate. Capsule wall single- layered, cells of the upper part with annular bands. Capsule with definite lid. Spores spherical, more or less muricate. Elaters fusiform tri-spiral.

Cyathodium tuberosum Kash. New Phytol. **Vol. XII,** p. 210 (1914) (Pl. VI-D).

Plants are dioecious, delicate, yellowish, or greenish and fluorescent. Male plants are smaller than female ones. Female plants grow by repeated dichotomy forming a fan- shaped structure; about 5-7 mm long and 15 mm broad. Epidermal cells are with chloroplasts. Ventral scales are not conspicuous, as they are green coloured due to presence of chloroplasts in them. Many sporangia are developed on the margins enclosed by involucres not projecting beyond the margins of the thallus. Capsule is circular with thick walls having annular bands. Spores are black, spinous, 40- 45 μ in diameter. Elaters are yellow, few in number, closely tri-spiral.

Habitat : On moist shady places, along the railway tunnels.

Locality : Purandar, Lonawala, Khandala, and Mahabaleshwar.

Date of Collection : August, 2009; September, 2010 and August 2011.

Family- Ricciaceae

Plant body dichotamously branched. Pores absent. Archegonia immersed singly in the cavities on the dorsal surface. Sporangium without foot or seta, dehiscing by the decay of the capsule wall. Elaters absent.

h) Genus - *Riccia* L.

Riccia L. Sp. Pl. p. 1138 (1753).

The thallus is dichotamously branched, terrestrial or more rarely floating on water. Dorsal surface usually green generally with a distinct median furrow. Ventral scales usually in one row at the apex, hyaline or purple. Rhizoids are of two types, smooth and tubericulate. In *Riccia fluitans*, they are absent. Antheridia and archegonia scattered singly on the dorsal surface. Involucres absent. Archegonia usually purple at the tip, neck occasionally projecting. Capsule sessile immersed in the midrib, without foot and seta. Calyptra is persistent. Spores are large tetrahedral, brown to black.

Riccia discolor L. Pugill.4: 1, 1832. (Pl. VI-E).

Plants are dioecious, once or twice forked. They are closely creeping, 5 mm long and 2 mm broad having long-linear lobes Dorsal surface is green. Air spaces are narrow, slit like. Epidermal cells are hyaline, thin walled. Ventral surface is purple, entire, bent downwards. Scales are small, semilunar, distant, purple or hyaline, bent over the margin. Antheridia lie in median row. Spores are tetrahedral.

Habitat : On moist condition.
Locality : Kas;Satara.
Date of Collection : September, 2010.

Riccia fluitans L. (Pl. VI-F).

The aquatic plants are floating on stagnant or slow running water. The thallus is dichotomously branched. Thalli are 35 mm long and 1 to 2 mm broad, thick and spongy. Segments are divergent, long linear, apex emarginated with a groove near the apex. Ventral side without scales and rhizoids. Cross section of the thallus shows parenchyma in many layers. In fertile plants, capsule forms a spherical protuberance on the ventral side. Spores are brownish yellow, translucent, margins broad.

Habitat : On bank of stagnant or slow running water.
Locality : Kas;Satara.
Date of Collection : September, 2010.

C. Physico-chemical characteristics of associated soil.

Soil composed of mineral matter together with small amount of organic matter, addition with number of organisms. It is a seat of greater chemical and biological activities. Soil microbes play an important role in organic matter decomposition and nutrient cycling (Bates and Farmer, 1990). Physical characteristics of thalloid liverworts associated soil are studied for their colour, texture, structure and constitution analysis.

Physical characteristics :

Soil colour -

Table. 1 : Colour of associated soils of thalloid liverworts.

Locality	Colour of the soil particles
Rajgad	Gray
Purandar	Deep black
Sinhagad	Reddish brown
Rayreshwar	Reddish brown
Kas;Satara	Late rite
Lonawala	Black
Khandala	Black
Panhala	White colour, Late rite
Mahabaleshwar	Yellow brown
Pachgani	Yellow brown
Shivthargad	Brown
Rohideshwar	Reddish brown
Raigad	Reddish brown

Soil texture:

Soil texture and their results by mechanical analysis are predicted in Tables. 2

Table. 2 A) Mechanical composition of thalloid liverworts associated soil (in %).

Layer	Rajgad	Purandar	Sinhagad
Clay	30	40	30
Silt	15	16	33
Fine sand	10	20	22
Coarse sand	45	34	15
Textural class	Sandy clay loam	Loamy sand	Silty clay loam

Table. 2 B) Mechanical composition of thalloid liverworts associated soil (in %).

Layer	Rayreshwar	Kas-Satara	Lonawala
Clay	35	28	42
Silt	15	22	13
Fine sand	25	35	10
Coarse sand	25	15	35
Textural class	Coarse sand	Loamy sand	Silty clay

Table. 2 C) Mechanical composition of thalloid liverworts associated soil (in %).

Layer	Khandala	Panhala	Mahabaleshwar
Clay	20	20	37
Silt	18	30	23
Fine sand	32	25	18
Coarse sand	40	25	12
Textural class	Silty clay	Loamy coarse sand	Clay loam

Sandy clay loam, loamy sand, silty clay loam, coarse sand, loamy sand, silty clay, silty clay, loamy coarse sand and clay loam soils reported subsequently from studied localities.

Soil structure: Liverworts associated soil and their structure are documented in Table. 3

Table. 3 : Soils structure classification from different localities of Western Ghats, Maharashtra.

Locality	Size of soil particles	Shape and Nomenclature
Rajgad	3-5 mm	Platy, Platy.
Purandar	1- 3 mm	Platy, Squamose.
Sinhagad	< 5 mm	Cubic, Granular.
Rayreshwar	1- 3 mm	Platy, Squamose.
Kas; Satara	< 5 mm	Cubic, Granular.
Lonawala	1- 3 mm	Platy, Squamose.
Khandala	1- 3 mm	Platy, Squamose.
Panhala	< 5 mm	Cubic, Granular.
Mahabaleshwar	1- 3 mm	Cubic, Granular.
Pachgani	1- 3 mm	Cubic, Granular.
Shivthargad	< 5 mm	Platy, Platy.
Rohideshwar	< 5 mm	Platy, Squamose.
Raigad	< 5 mm	Cubic, Granular.

Soil constitution:

Some soil samples from Panhala locality are with lime, and silica particles and Sinhagad regions soil samples indicates the iron oxides with silica. Remaining of all localities soil samples are without any constitution.

New growth: New growth formation in form of streaks and patches with lime nodules through physicochemical reactions accumulate in the soil have been seen at Panhala locality.

Determination of soil reaction (pH) and Electrical conductivity (E.C.): pH and electrical conductivity results of soil are summarized in Table Nos. 4 to 9.

Chemical characteristics: (Table Nos. 4 to 9)

The thalloid liverworts associated soil analysis from each localities of Western Ghats of Maharashtra, are depicted in Table Nos. 4 to 9. They includes available nitrogen (N), phosphorous (P), and potassium (K), Na, Ca, organic matter (C); electrical conductivity and pH.

The rating of soil testing values of essential nutrients from different localities of Western Ghats of Maharashtra are compared with ICAR (2006) rating chart of soil testing values and our observations are made for variation of basal, middle and high altitudinal values.

Rating Chart of Soil testing values of essential nutrients:*

Sr. No.	Nutrients	Compounds (Units)	Rating		
			Low	Medium	High
1	C	Organic Carbon (%)	<0.4	0.4-0.75	>0.75
2	N	Alkaline $KMnO_4$(kg/ha)	<280	281-560	>560
3	P	Olsen's (kg/ha)	<12.5	12.5-25	>25
4	K	Ammonium Acetate (kg/ha)	<135	135-335	>335
5	Ca	Ammonium Acetate cmol (P^+)/kg	<1.5	-	>1.5
6	Mg	Ammonium Acetate cmol (P^+)/kg	<1.0	-	>1.0
7	Zn	DTPA extractable (mg/kg)	<6.0	6.0-1.2	>1.2
8	Fe	DTPA extractable (mg/kg)	<4.5	4.5-9.0	>9.0
9	Mn	DTPA extractable (mg/kg)	<3.5	3.5-7.0	>7.0
10	Cu	DTPA extractable (mg/kg)	<0.2	0.2-0.4	>0.4
11	B	Hot water soluble (kg/ha)	<0.5	0.5-1.0	>1.0
12	Mo	Ammonium oxalate extractable (pH 3.3) (mg/ha)	<0.2	0.2-0.4	>0.4
13	S	0.01 M $CaCl_2$ (kg/ha)	<22.4	22.4-35	>35

*(Handbook of Agriculture, Indian Council of Agricultural Research, New Delhi, 2006).

Therefore, as per ICAR (2006) rating values of essential nutrients, we summarize the findings that the K, Ca and C values are less to medium than average values at all studied localities.

The soil testing values of essential nutrients from different localities of Western Ghats of Maharashtra based on ICAR (2006) rating chart of soil testing values are as follows -

Locality	Altitude														
	Basal					Middle					High				
Rajgad	M	L	G	G	L	L	L	G	G	L	G	L	G	G	L
Purandar	M	L	L	G	L	M	L	G	G	L	L	L	G	G	G
Sinhagad	L	L	G	M	G	L	M	G	G	G	L	M	G	L	G
Rayreshwar	L	L	G	G	G	L	L	M	L	G	L	M	G	M	G
Kas;Satara	L	L	L	L	G	L	L	M	G	G	L	M	G	M	G
Lonawala and Khandala	L	L	M	G	L	G	M	M	L	G	L	L	G	G	L
Panhala	L	L	M	G	G	M	L	G	G	G	G	L	G	G	G
Mahabaleshwar	L	M	M	L	G	L	L	G	G	G	G	L	G	G	G
Pachgani	L	L	L	L	G	L	L	L	M	G	L	M	M	L	G
Shivathargad	M	L	M	G	L	L	M	M	M	G	L	L	G	G	L
Rohideshwar	L	L	M	G	L	G	L	G	G	L	G	L	G	G	L
Raigad	L	L	M	G	L	M	L	G	G	L	L	L	G	G	G

Where values : L - Value for less, M - Value for medium, G - Value for greater.

The soil testing greater than average values of essential nutrients at different altitudes from localities of Western Ghats of Maharashtra based on ICAR (2006) rating chart of soil testing values are as follows –

Locality	Altitudinal Essential Nutrients Elements		
	Basal	Middle	High
Rajgad	K, C	K, C	N, K, C
Purandar	C	K, C	K, C, Ca
Sinhagad	K, Ca	K, C, Ca	K, Ca
Rayreshwar	K, C, Ca	Ca	K, Ca
Kas,Satara	Ca	C, Ca	K, Ca
Lonawala and Khandala	C	N, Ca	K, C
Panhala	C, Ca	K, C, Ca	N, K, C, Ca
Mahabaleshwar	Ca	K, C, Ca,	N, K, C, Ca
Pachagani	Ca	Ca	Ca
Shivathargad	C	Ca	K, C
Rohideshwar	C	N, K, C	N, K, C
Raigad	C	K, C	K, C, Ca

Where, N - Nitrogen, P - Phosphorus, K - Potassium, C - Carbon and Ca - Calcium.

Rajgad locality showed one and half to two times greater than average values of 'K' and 'C' at basal and middle altitude and 'N' at high altitude. Other altitudinal N, P, K values are less.

Purandar locality showed two to three times greater 'C' at all altitudes, along with two times greater 'K' at middle altitude and greater 'Ca' at high altitude. N and P are medium to less at all localities.

Sinhagad regions values interestingly indicates the one and half to two times greater 'K' along with three times greater 'Ca' from all altitudes. Carbon is greater at middle altitude only. N, P and C values are medium to less at all altitudes.

Rayereshwar regions showed two to five times greater 'K' and 'Ca' at basal and high altitude, respectively and greater 'C' only at middle. Kas region showed one, one and half and two times greater values of K, Ca and C at basal, middle and high altitude. N, P, K values are less to medium at all altitudes.

Lonawala and Khandala locality showed two to three times greater values of 'C' at basal, N and Ca at middle; K, and Cat high altitude. N, P, K values are less to medium at all altitudes. Value rating reduced to half for 'C' at middle and for 'N' at high altitude. Panhala locality indicates two to three times greater values of 'C' and 'Ca' from all altitudes, 'K' and 'N' four times greater at middle and high altitudes, respectively.

Mahabaleshawar regions soil samples showed one, two and three times greater 'Ca' at basal, middle and high altitudes, respectively. Potassium (K) is two times greater and carbon is four times greater at middle and high altitude along with greater values of 'N' at high altitude. Phosphorus (P) values are medium at all altitudes whereas 'N' and 'P' values are less to medium at basal and middle altitude. Pachagani regions soil samples indicates 'Ca' with one to two times greater values at all altitudes. N, P, K and C less to medium from all localities.

Basal and high altitudinal areas soil from Shivathargad indicates one to three times greater 'C' values, two times greater 'Ca' values at middle and two times greater 'K' at high altitude. N, P and K values are medium to less at all altitudes.

Rohideshwar regions soil samples showed two to three times greater values of 'C' at basal, middle and high altitudes, whereas greater 'N' and two times greater 'K' values at middle and high altitudes. N, P and Ca values are medium at other altitudes. Lastly Raigad regions soil samples indicates two to four times greater values than average values of 'C' at all altitudes and 'K' values three times greater at middle and high altitude.

C, Table No. 4: Nutrient elements and E. C., pH values of thalloid liverworts associated soil from **Rajgad** and **Purandar** regions of Western Ghats, Maharashtra.

Altitude	Genera	N *	P*	K*	C %	Na**	Ca %	E.C.***	pH
	I) Rajgad								
Basal	*Plagiochasma simulensis* Kash.	425.37	3.59	455.84	2.73	7.00	0.10	0.20	5.55
Middle	*Targionia hypophylla* (Mich) L.	561.94	4.21	654.08	2.41	1.40	0.27	0.13	5.77
High	*Cyathodium tuberosum* Kash.	196.43	2.87	501.76	3.31	1.70	0.40	0.15	5.95
	II) Purandar								
Basal	*Plagiochasma articulatum* Kash.	307.32	4.22	110.88	2.62	7.10	0.12	0.10	7.26
Middle	*Plagiochasma simulensis* Kash.	335.55	1.39	347.02	3.37	7.80	0.60	0.53	7.01
High	*Fossombronia indica* St.	188.16	3.40	338.24	2.25	7.90	2.00	0.18	7.24

* Values are expressed in Kg/ha

** Values in M.E./L

*** Values in $dS.m^{-1}$

Table No. 5 : Nutrient elements and E. C., pH values of thalloid liverworts associated soil from **Sinhagad** and **Rayreshwar** of Western Ghats, Maharashtra.

Altitude	Genera	N *	P*	K*	C %	Na**	Ca %	E.C.***	pH
	III) Sinhagad								
Basal	*Plagiochasma articulatum* Kash.	147.0	13.06	498	0.61	1.2	4.70	0.41	7.79
Middle	*Exormotheca tuberifera* Kash.	183.0	14.40	873	0.76	0.90	4.70	6.10	5.94
High	*Sewardiella tuberifera* Kash.	62.3	14.02	742	0.26	0.97	4.23	2.28	6.51
	IV) Rayreshwar								
Basal	*Plagiochasma simulensis* Kash.	253.0	11.23	1164	1.05	1.30	7.52	1.55	7.72
Middle	*Riccia discolar* L.	62.6	13.62	296	0.26	1.22	3.29	11.20	6.52
High	*Exormotheca tuberifera* Kash.	197.0	11.87	758	0.69	0.98	4.02	1.80	6.43

* Values are expressed in Kg/ha

** Values in M.E./L

*** Values in $dS.m^{-1}$

Table No. 6 : Nutrient elements and E. C., pH values of thalloid liverworts associated soil from **Kas;Satara** and **Lonawala** regions of Western Ghats, Maharashtra.

Altitude	Genera	N *	P*	K*	C %	Na**	Ca %	E.C.***	pH
	V) Kas;Satara								
Basal	*Riccia fluitans* L.	69.80	12.0	120.00	0.29	0.96	2.82	17.72	5.93
Middle	*Cyatodium tuberosum* Kash.	186.03	2.82	314.10	4.27	1.83	1.67	0.32	6.61
High	*Fossombronia indica* St.	149.00	14.20	473.00	0.62	0.61	3.76	1.00	6.30
	VI) Lonawala & Khandala								
Basal	*Asterella angusta* St.	255.37	4.22	228.21	3.91	4.78	0.73	0.29	6.55
Middle	*Plagiochasma simulensis* Kash.	55.40	15.03	207.00	0.23	1.00	3.76	7.64	7.01
High	*Cyatodium tuberosum* Kash.	171.36	1.29	527.74	3.46	1.19	0.93	0.17	6.20

* Values are expressed in Kg/ha
** Values in M.E./L
*** Values in $dS.m^{-1}$

Table No. 7 : Nutrient elements and E. C., pH values of thalloid liverworts associated soil from **Panhala** and **Mahabaleshwar** regions of Western Ghats, Maharashtra.

Altitude	Genera	N *	P*	K*	C %	Na**	Ca %	E.C.***	pH
	VII) Panhala								
Basal	*Plagiochasma simulensis* Kash.	120	13.06	316	0.50	0.95	2.82	20.20	6.80
Middle	*Plagiochasma articulatum* Kash.	226	12.22	1120	0.94	2.80	4.23	0.99	7.35
High	*Cyatodium tuberosum* Kash.	174	3.29	566	3.45	1.32	2.95	0.24	6.82
	VIII) Mahabaleshwar								
Basal	*Plagiochasma articulatum* Kash.	173	16.00	240	0.30	0.34	2.12	16.13	5.84
Middle	*Plagiochasma simulensis* Kash.	133	12.20	457	0.87	3.60	5.21	0.89	7.35
High	*Targionia hypophylla* (Mich) L.	147	9.10	630	4.12	1.91	3.15	0.41	6.26

* Values are expressed in Kg/ha

** Values in M.E./L

*** Values in $dS.m^{-1}$

Table No. 8 : Nutrient elements and E. C., pH values of thalloid liverworts associated soil from **Pachgani** and **Shivthargad** regions of Western Ghats, Maharashtra.

Altitude	Genera	N *	P*	K*	C %	Na**	Ca %	E.C.***	pH
	IX) Pachgani								
Basal	*Plagiochasma simulensis* Kash.	83.30	5.60	86.00	0.26	0.64	3.29	7.20	6.17
Middle	*Asterella angusta* St.	164.00	12.12	114.10	2.17	0.81	1.62	0.22	3.53
High	*Plagiochasma articulatum* Kash.	199.00	13.20	293.00	0.52	0.81	2.76	0.58	6.68
	X) Shivthargad								
Basal	*Cyatodium tuberosum* Kash.	435.77	2.25	281.00	1.90	3.80	0.93	0.19	6.50
Middle	*Plagiochasma articulatum* Kash.	33.30	16.00	207.00	0.56	1.80	2.68	1.64	7.00
High	*Plagiochasma simulensis* Kash.	271.30	1.90	527.74	2.16	1.90	0.73	0.77	6.67

* Values are expressed in Kg/ha

** Values in M.E./L

*** Values in $dS.m^{-1}$

Table No. 9 : Nutrient elements and E. C., pH values of thalloid liverworts associated soil from **Rohideshwar** and **Raigad** regions of Western Ghats, Maharashtra.

Altitude	Genera	N *	P*	K*	C %	Na**	Ca %	E.C.***	pH
	XI) Rohideshwar								
Basal	*Targionia hypophylla* (Mich) L.	295.30	3.79	265.80	1.70	7.43	0.40	0.80	6.75
Middle	*Plagiochasma simulensis* Kash.	611.14	2.41	548.00	2.80	1.45	0.67	0.53	7.10
High	*Plagiochasma articulatum* Kash.	665.32	2.80	501.66	3.50	1.77	0.80	0.95	6.55
	XII) Raigad								
Basal	*Plagiochasma articulatum* Kash.	227.34	4.62	155.80	2.79	7.69	0.24	0.60	5.26
Middle	*Cyathodium tuberosum* Kash.	385.77	1.98	386.00	3.67	6.80	0.89	0.33	6.81
High	*Plagiochasma simulensis* Kash.	182.67	2.60	356.27	1.48	7.00	2.45	0.98	6.29

* Values are expressed in Kg/ha
** Values in M.E./L
*** Values in $dS.m^{-1}$

D. Biological characteristics

Identification of fungi: (Table. 5-D, 1) (Pl. VII-A to IX-F).

The present investigation deals with the mycoflora of liverworts associated soils from different localities of Western Ghats, Maharashtra. Both fungal as well as bacterial colonies are isolated on media in petridishes and bacterial and fungal identifications made by routine method (Mehrotra and Aneja, 1998; Ainsnorth, 1973; Alexopoulos *et al.*, 2002).

A total number of 10 fungal genera are identified with their variations occurred as per altitudinal areas. *Aspergillus niger, Penicillium notatum, Saccharomyces cerevisiae, Trichoderma harzianum, Fusarium oxysporum, Mucor mucedo, Theilavia basicola, Alternaria* sp., *Chaetomium* sp., and *Glomus fasciculatum* mycorrhizae are reported. *A. niger, P. notatum* and *T. harzianum* are most common fungi. Biological characteristics including mycoflora from thalloid liverworts associated soil are -

Rajgad : *Aspergillus, Penicillium, Saccharomyces, Thielavia, Glomus.*

Purandar :*Aspergillus,Fusarium, Penicillium, Saccharomyces, Trichoderma. Chaetomium, Mucor.*

Sinhagad : *Aspergillus, Glomus.*

Rayreshwar : *Mucor, Penicillium, Glomus.*

Kas; Satara : *Aspergillus, Penicillium, Chaetomium, Mucor.*

Lonawala : *Aspergillus, Saccharomyces.*

Khandala : *Aspergillus, Penicillium, Alternaria, Glomus.*

Panhala : *Aspergillus, Penicillium, Trichoderma, Chaetomium.*

Mahabaleshwar: *Aspergillus, Penicillium, Saccharomyces, Glomus.*

Pachgani : *Aspergillus, Saccharomyces.*

Shivthargad : *Aspergillus, Penicillium.*

Rohideshwar : *Aspergillus, Penicillium, Trichoderma.*

Raigad : *Aspergillus, Penicillium.*

5-D, **Table No. 1 :** Mycoflora diversity in associated soil of thalloid liverworts from Western Ghats of Maharashtra.

Locality	Altitude	Name of the species	Mycoflora diversity*									
			A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Rajgad	Basal	*Plagiochasma simulensis* Kash.	+	+	–	–	–	–	–	–	–	–
	Middle	*Targionia hypophylla* (Mich) L.	–	–	+	+	–	–	–	–	–	–
	High	*Cyatodium tuberosum* Kash.	–	–	–	–	–	–	–	+	–	–
Purandar	Basal	*Plagiochasma articulatum* Kash.	+	+	+	–	–	–	–	–	–	–
	Middle	*Plagiochasma simulensis* Kash.	+	–	–	–	–	+	+	–	+	–
	High	*Sewardiella tuberifera* Kash.	+	–	+	–	–	–	–	–	–	–
		Fossombronia indica St.	–	+	+	–	–	–	–	–	–	–
Sinhagad	Basal	*Plagiochasma simulensis* Kash.	+	–	–	+	–	–	–	–	–	–
		Sewardiella tuberifera Kash.	–	–	+	–	–	–	–	–	–	–
	Middle	*Exormotheca tuberifera* Kash.	–	–	–	–	–	–	–	–	–	–
	High	*Sewardiella tuberifera* Kash.	–	–	–	–	–	–	–	–	–	–
		Plagiochasma articulatum Kash.	–	–	–	+	–	–	–	–	–	–

Present (+), Absent (-).

*A - *Aspergillus niger*, B - *Penicillium notatum*, C - *Saccharomyces cerevisiae*,
D - *Glomus fasciculatum*, E - *Trichoderma harzianum*, F - *Fusarium oxysporum*,
G - *Mucor mucedo*, H - *Thielavia basicola*, I - *Chaetomium* sp.,
J - *Alternaria* sp.

Locality	Altitude	Name of the species	Mycoflora diversity*									
			A	B	C	D	E	F	G	H	I	J
Rayreshwar	Basal	*Plagiochasma simulensis* Kash.	–	–	–	–	–	–	+	–	–	–
		Plagiochasma articulatum Kash.	–	+	–	–	–	–	–	–	–	–
	Middle	*Asterella angusta* St.	–	–	–	–	–	–	–	–	–	–
	High	*Exormotheca tuberifera* Kash.	–	–	–	–	–	–	–	–	–	–
Kas;Satara	Basal	*Targionia hypophylla* (Mich) L.	+	+	–	–	–	–	–	–	–	–
	Middle	*Cyatodium tuberosum* Kash.	+	+	–	–	–	–	–	–	+	–
	High	*Fossombronia indica* St.	+	+	–	–	–	–	–	–	+	–
		Plagiochasma articulatum Kash.	+	+	–	–	+	–	+	–	–	–
Lonawala; Khandala	Basal	*Asterella angusta* St.	+	+	–	+	–	–	–	–	–	–
	Middle	*Plagiochasma simulensis* Kash.	+	–	–	+	–	–	–	–	–	–
	High	*Cyatodium tuberosum* Kash.	–	–	–	–	–	–	–	–	–	+

Present (+), Absent (-).

*A - *Aspergillus niger*, B - *Penicillium notatum*, C - *Saccharomyces cerevisiae*, D - *Glomus fasciculatum*, E - *Trichoderma harzianum*, F - *Fusarium oxysporum*, G - *Mucor mucedo*, H - *Thielavia basicola*, I - *Chaetomium* sp., J - *Alternaria* sp.

Locality	Altitude	Name of the species	Mycoflora diversity*									
			A	B	C	D	E	F	G	H	I	J
Panhala	Basal	*P. simulensis* Kash.	+	+	–	–	–	–	–	–	–	–
	Middle	*Plagiochasma articulatum* Kash.	+	+	–	–	–	–	–	–	+	–
	High	*Cyatodium tuberosum* Kash.	+	–	–	+	–	–	–	–	–	–
		Targionia hypophylla (Mich) L.	–	–	–	–	+	–	–	–	–	–
Mahabaleshwar	Basal	*Plagiochasma articulatum* Kash.	+	+	–	+	–	–	–	–	–	–
	Middle	*P. simulensis* Kash.	+	–	+	+	–	–	–	–	–	–
	High	*Targionia hypophylla* (Mich.) L.	+	–	–	–	–	–	–	–	–	–
Raigad	Basal	*Plagiochasma articulatum* Kash.	+	–	+	–	–	–	–	–	–	–
	Middle	*Cyathodium tuberosum* Kash.	+	+	–	–	–	–	–	–	–	–
	High	*Plagiochasma articulatum* Kash.	–	+	–	–	–	–	–	–	–	–

Present (+), Absent (-).

*A - *Aspergillus niger*, B - *Penicillium notatum*, C - *Saccharomyces cerevisiae*,
D - *Glomus fasciculatum*, E - *Trichoderma harzianum*, F - *Fusarium oxysporum*,
G - *Mucor mucedo*, H - *Thielavia basicola*, I - *Chaetomium* sp.,
J - *Alternaria* sp.

PLATE - VII

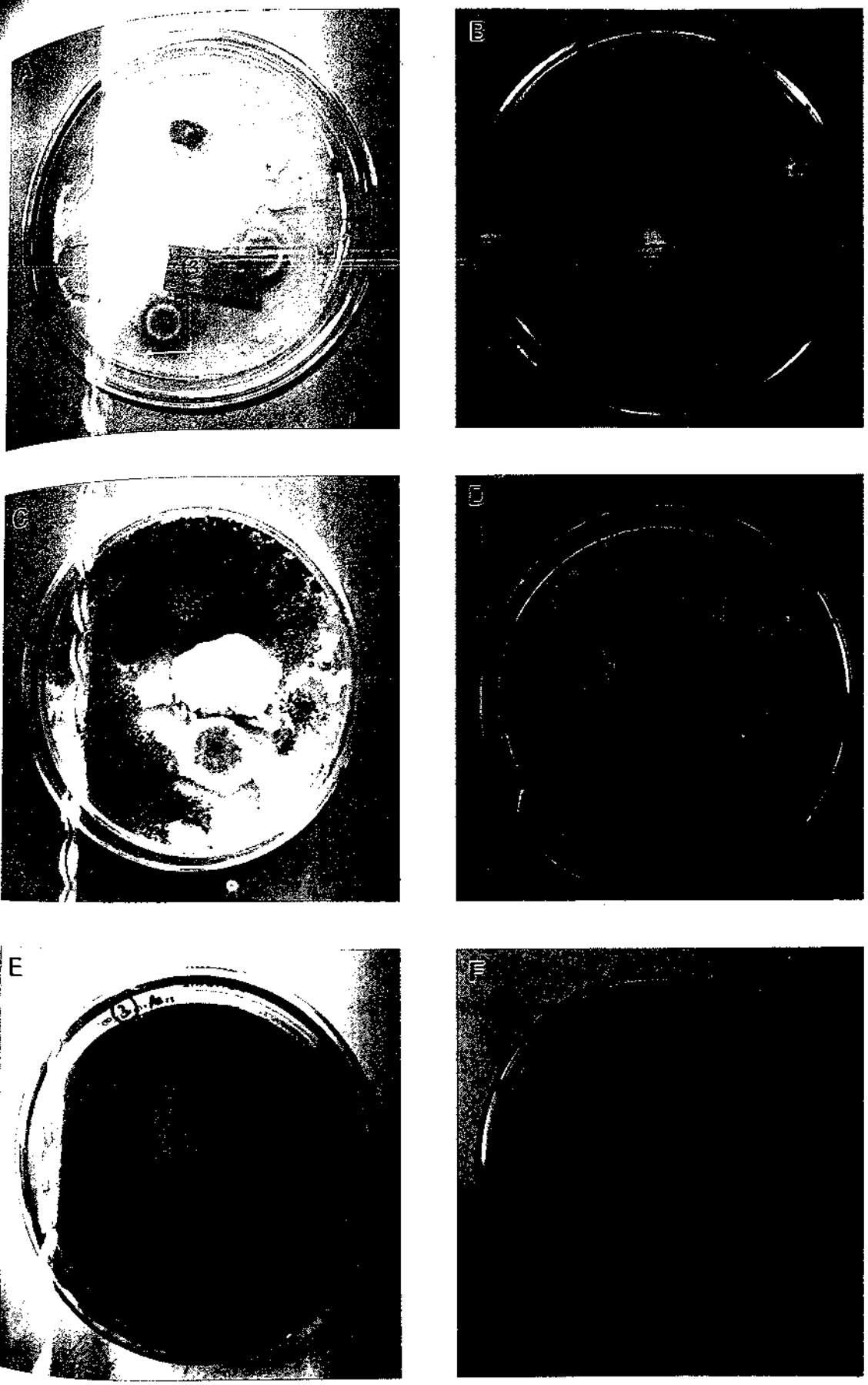


Photo Plates of isolated mycoflora: **A.** *Fusarium oxysporum* Schlechtendahl.; B. *Saccharomyces cerevisiae* Hansen.; C. *Aspergillus* sp.; D. *Mucor mucedo* ; E. *Chaetomium* sp.; F. *Alternaria* sp.

PLATE - VIII

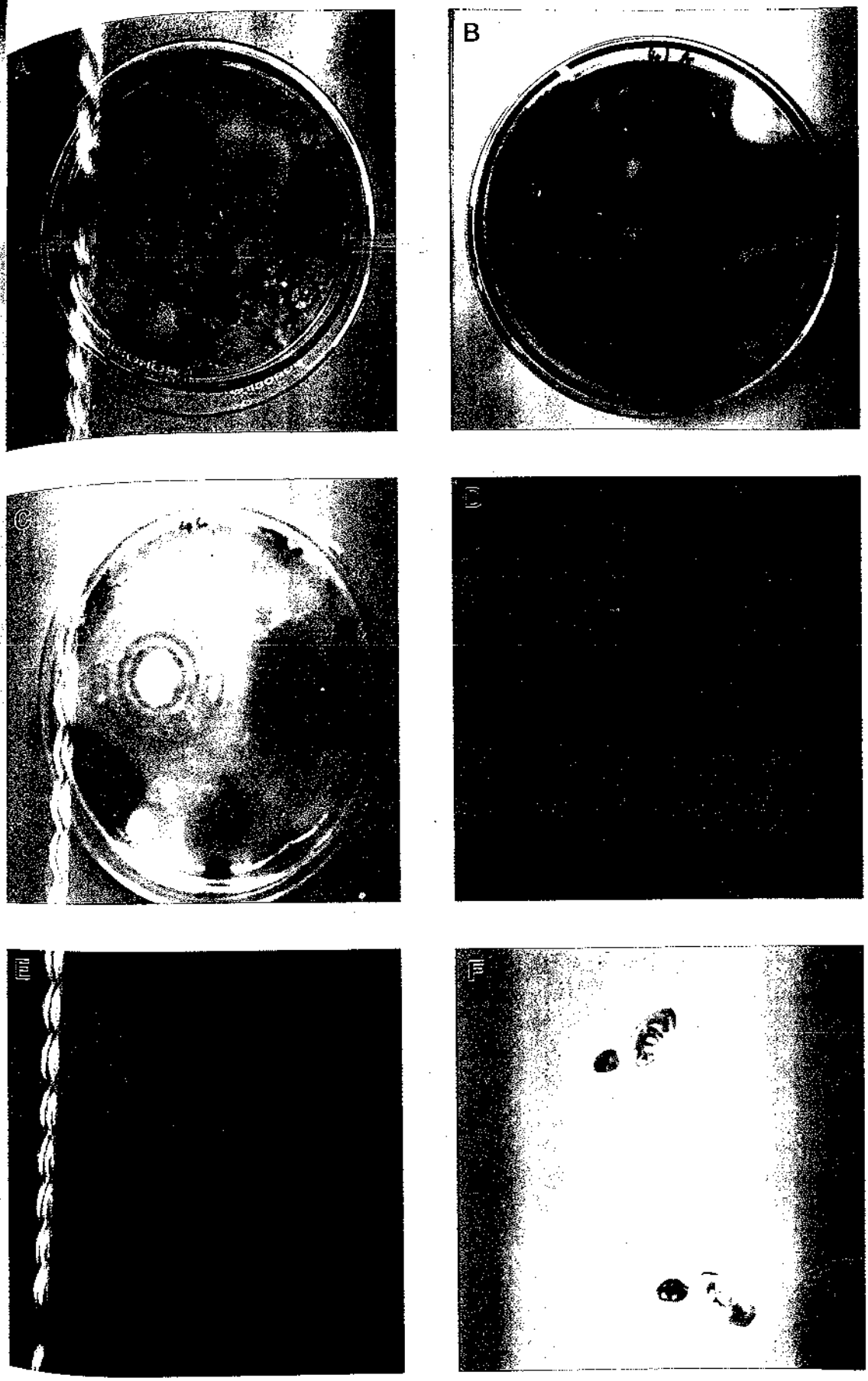
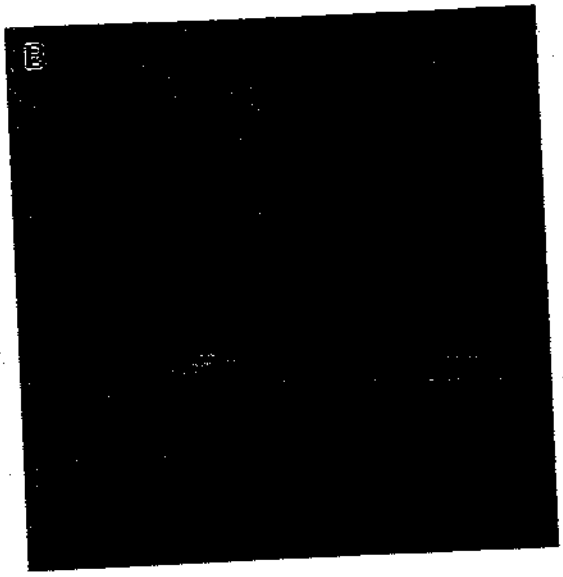
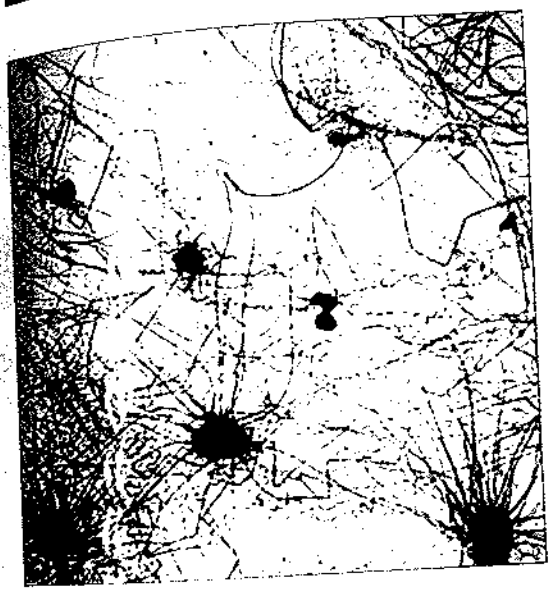
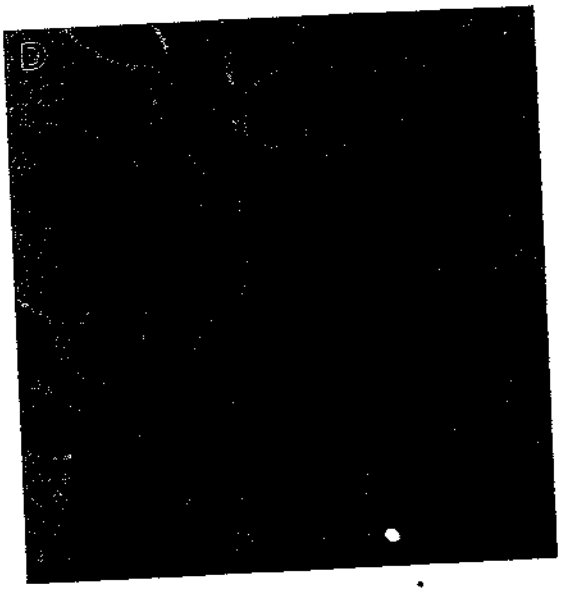
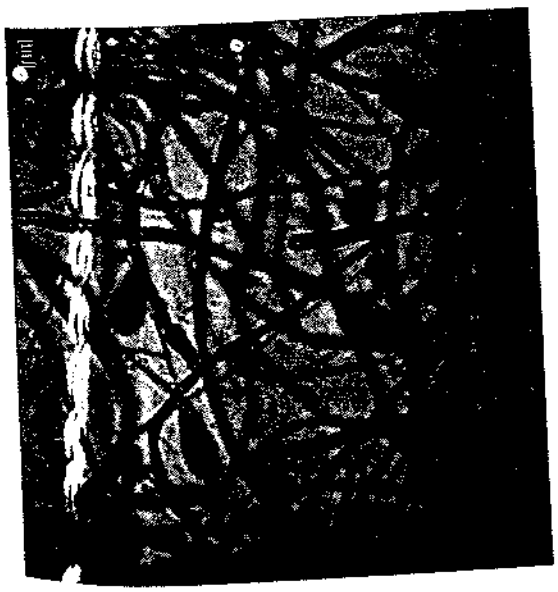


Photo Plates of isolated mycoflora: **A.** *Aspergillus niger* Van. Tieghem.; B. *Trichoderma harziarum* **Rifai.;** C. *Penicillium notatum* **Westling.** Photo Plates of identified fungal **genera:** D. *Aspergillus niger* Van. Tieghem.; E. *Penicillium notatum* Westling; **F. *Saccharomyces cerevisiae*** Hansen.









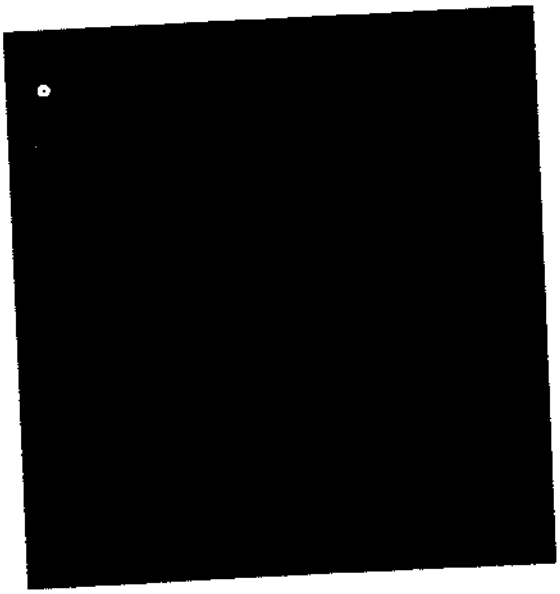


Photo Plates of identified fungal genera: A. *Mucor mucido;* B. *Glomus fasciculatum;* C. *Chaetomium* sp.; D. *Theilavia basicola;* E. *Alternaria* sp.; F. *Fusarium oxysporum.*

Isolated mycoflora from liverworts associated soil are depicted in Plates. Fungal genera *viz. Alternaria, Aspergillus, Fusarium, Chaetomium, Mucor, Penicillium, Saccharomyces, Thielavia, Trichoderma* and *Glomus* mycorrhiza are identified from Rajgad, Purandar, Sinhagad, Rayreshwar, Kas; Satara, Lonawala, Khandala, Panhala, Mahabaleshwar, Pachgani, Shivthargad, Rohideshwar and Raigad localities of Western Ghats, Maharashtra. *Penicillium*, *Aspergillus*, *Trichoderma, Chaetomium* fungal genera and *Pseudomonas, Bacillus* bacterial genera isolated and identified from *Fossombroniaindica* St. soil at Panhala. Fungi *Chaetomium*, *Mucor* and bacteria *Pseudomonas* reported in soil at Purandar. *Penicillium*, *Alternaria, Aspergillus* and *Glomus* from *Cyatodium tuberosum* Kash. soil at Lonawala. *Penicillium, Aspergillus, Fusarium* and *Trichoderma* reported from Purandar. *Chaetomium, Aspergillus* and *Mucor mucedo* in *Plagichasma* L.et L. soil at Kas; Satara. *Trichoderma, Chaetomium, Penicillium* and *Aspergillus* from *P. articulatum* Kash. at Panhala. *Glomus* in *Targionia hypophylla* Kash. soil at Sinhagad. *Penicillium* and *E. coli* isolated from soil at Rayreshwar. *Aspergillus* and *Chaetomium* and bacteria *Pseudomonas* reported in *Cyathodium tuberosum* Kash. liverworts associated soil at Kas; Satara region.

Majority of fungi are saprophytic and few parasitic. Fungi are divided into four. Classes - Phycomycetes, Ascomycetes, Basidiomycetes and Hypomycetes. Numerous genera of fungi found in soil, the most common *Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Trichoderma* and *Zygorhynchgus*. A few species belonging to genera *Absidia, Acrostalagus, Botrytis, Cephalosporium, Monilia, Oospora, Saccharomyces, Spicaria* and *Verticillium* but, they are less abundant.

Identification of bacteria:

The common bacterial genera are *Bacillus* and *Pseudomonas* isolated from associated soil. Bacteria form the most abundant group of microorganisms in soil. Bacteria belongs to thallophytic group of very primitive organisms. They classified into

groups: *Cocci*, *Bacilli* and *Spirilli*. Taxonomically divided into number of orders from which three Pseudomonadales, Eubacteriales and Myxobacteriales important as their presence and activitics in soil are concerned. Pseudomonadales includes the genera *Bacillus* (Bacillaceae), *Pseudomonas* (Pseudomonadaceae), and *Escherichia* (Enterobacteriaceae) are most abundant.

Fungal flora of varies with the nature of soil condition. These are more abundant in acid soils than in neutral or alkaline soils and the number of fungi is usually low in summer, and high in monsoon (Waksman, 1944). Variegated colours are due to phenomenon of oxidation and reduction of iron and manganese compounds and their hydrated products and are an indicator of geographical and pedological origin of the soil (Kamat, 1959).

The pH difference most likely reflect the difference in uptake ability of calcium ions and other nutrients. From alkaline and neutral soils, some thalloids prefer acidic and some prefer calcareous soil (Nakanishi and Hiroaka, 1981). Soil science deals with the aspects of nutrients availability, microbial activity, moisture, Physico-chemical and biological characteristics of associated soil. Some leafy liverworts *Scapantia undulata* and *Nardia compressa* occurs mostly in pH ranges 5.2 to 5.8 whereas, moss *Fontalis* occurs at pH 5.6 to 6.2, and *Riccia fluitans* L. has better growth in the range of pH 3-5 than other levels (Omerod and Wade, 1987).

Environmental factors like soil acidity and the amount of organic matter present influences the fungal population considerably. In neutral and alkaline soils where, bacteria and actinomycetes are more active. Soil nutrients and their availability depends up on the type of species and litter which provide nutrient supply. *Hylocomium* species obtain Ca ions from $CaCo_3$ in soil (Bates and Farmer, 1990). Due to very thin leaves and lack of cuticle and stoma in bryophytes they are sensitive to pH and water availability (Kellner and Wiebull, 1998). Bryophytes are sensitive to pH and water availability, and

would probably respond to factors associated with tree species. Effect of litter is probably one of the most important factor regulating bryophytes species composition in forests (Xiong and Nilsson, 1999). The relative humidity change in forests will affects the occurrence and performance of bryophytes (Hazell and Gustfsson, 1999). Physical and chemical nature of soil influences the air, moisture and food supply of the microorganisms and affects the nature, type and abundance of the microbial population. Environmental factors like moisture, temperature, light, air, reaction, food and energy supply, nature of soil influencing their activity where organisms live. Organisms showed antagonistic and associative effects themselves. Also, the nature of vegetation influences the soil microorganisms (Weibull, 2000).

Thalloid liverworts *Coenocephalum conicum* and *Pellia neesiana* grow well in high N and Ca concentration (Wilkinson *et al.*, 2005). Our results of elements analysis from different altitudinal areas could differ and indicates the variable pH ranges. The deep black and loamy sand contains a very large amount of fine particles, especially clay from Purandar, which makes it very suitable for growth of thalloids. On the other hand, late rite soil at Kas;Satara, red soil from Rayreshwar, black soil from Lonawala, and Khandala, brown at Sinhagad, gray at Rajgad and white soil samples at Panhala are observed.

Approximately 300 species of Ascomycetes appear to grow as obligates on bryophytes reported by Doebbeler (1997). On the contrary, Raspe and De Sloover, (1998) have suggested that the fungus *Mniaecia jungermanniae* lives exclusively on leafy liverworts in the Jungermanniales and might have achieved the first step toward mutualism. It is interesting note is that liverworts showed *Glomus* arbuscular mycorrhiza (AM) association. It has been known for a longtime that liverwort and hornworts form AM association. *Glomus claroideum* form symbiosis with the hornwort *Anthoceros punctatus* L. (Schubler, 2000). The symbiotic fungal associations with leafy liverworts

are investigated by Ingrid *et al.*, (2003). There is interesting association of bryophytes and fungi but very little attention has been paid by bryologists.

The AM fungus *Glomus* forming endo-symbioses with liverwort *Marchantia foliacea* was reported by Russell and Bulman, (2004). *Glomus fasciculatum* in associated soils of *Targionia hypophylla* (Mich) L., *Plagiochasma simulensis* Kash., and *P. articulatum* Kash. reported at Sinhagad. Analysis have needs to get awareness of threatening diverse microflora as early as possible. There was significant variation in physical and chemical characteristics of liverworts associated soils. *Aspergillus, Trichoderma, Penicillium*, and *Saccharomyces* are the dominant and common genera in analyzed soil. *Aspergillus* genus only adapted to different environmental conditions. *Trichoderma* indicating the disease suppressive characteristics of soil samples.

Alternaria and *Fusarium* are also reported in liverworts associated soils. These organisms are also able to produce secondary metabolites, have antibiotic activity. Lei *et al.* (2008) noticed that an endophytic fungus *Chaetomium fusiformae* from a liverwort *Scapania verrucosa*. Interesting thing is that *Scapania verrucosa* and its endophyte showed antifungal and antitumor activities. *C. fusiformae* has displayed a widal range of antimicrobial and antitumor activities, which are better than the host plant. These results could support the suggestion of endophytes as an alternative of the host for medicinal activity.

The altitudinal ranges (basal, middle and high) of Western Ghats, Maharashtra, have seem to affect the frequency and density of mycoflora. This isolated mycoflora varied in different areas of localities indicating soil quality variation. This analysis yielded 10 fungal genera from liverworts associated soils. Maximum density of fungal species noticed at basal altitude, but on the contrary high altitude showed lesser number of population.

E. Antimicrobial screening of thalloid liverworts:

Determination of antimicrobial activity by 'Disc diffusion assay' method. Different organic solvent extracts of *Fossombronia indica* St. from Purandar were screened for antimicrobial screening, separately. The results indicated that the greater inhibitory activity of ethanol extract against *S. aureus* (16 mm) whereas, minimum activity against *E. coli*, and *B. substilis* (7 mm) depicted in Table Nos. 1 to 2. The average zone of inhibition for ethanolic extracts is (7.5 mm). No antibacterial activity exhibits when extracted with aqueous except of *S. aureus*, remaining all extracts showed common antibiotic activity. From Panhala region plants ethanol extract has more antibiotic activity against *E. coli* (13 mm). It was found that zone inhibition of remaining extracts had varying antimicrobial activities against tested bacteria. The antibiotic amphicillin used as positive control, has more inhibitory activity against *B. substilis* (19 mm).From Sinhagad regions *Sewardiella tuberifera* Kash. used to screen antifungal and antibacterial activities. The results of screening were summarized in Table Nos. 3 to 4. *F. oxysporum* is very sensitive to *S. tuberifera* Kash. ethanol extract (10.5 mm) with diameter zone inhibition. Acetone extract showed greater antibacterial activity against *S. aureus* (17.5 mm). *B. substilis*, and *P. aeruginosa* has no sensitivity to any extract. Positive control nyastatin and amphicillin exhibits more antifungal and antibacterial activity against *F. moniliformae* (14.5 mm), and *S. aureus* (21 mm).

The results of antifungal and antibacterial activities of *Exormotheca tuberifera* Kash. extracts were summarized in Table Nos. 5 to 6. Only *A. niger* showed sensitivity to all organic solvents and aqueous extracts. Acetone extract showed maximum zone inhibition (15.6 mm). *Rhizopus stolonifer* sensitive to petroleum ether extract have lower inhibitory activity (7 mm). Antibiotic, nyastatin exhibits more activity against *F. oxysporum* (16 mm). Ethanol extract indicate that the greater antibacterial activity against *S. aureus* (13.5 mm), and lower activity against *B. substilis* (7 mm). *E. coli* only sensitive to acetone extract (15 mm). Antibiotic amphicillin give response to all bacterial

strains. Joshi *et al.*, (1990) observed previously that the effect of *Exormotheca tuberifera* extracts on against four bacterial strains *viz. Escherichia coli, Klebsiella pneumonae, Bacillus substilis*, and *staphylococcus aureus*. Antimicrobial activity of *Asterella angusta* St. extracts against the target organisms were summarized in Table Nos. 7 to 8. The *A. angusta* St. extracts from Lonawala regions used for antimicrobial screening. Among the solvent extracts, the ethanolic extract showed 14 mm inhibition, followed by acetone extract 12 mm, petroleum ether extract 9 mm, and aqueous extracts 8 mm of zone inhibition. Ethanol extracts exhibits the zone inhibitions whereas, lower inhibitions for aqueous extract. The conventional antibiotic nystatin are generally more active against *Rhizopus stolonifer* than other strains. In antibacterial testing, the ethanolic extract also showed maximum zone of inhibition i.e. 13 mm; followed by acetone (10 mm) for aqueous and petroleum ether extracts, respectively. The antibiotic amphicilin, more active against *S. aureus*. The cidal activity in *A. angusta* St. extracts, due to presence of bioactive compounds.

Antimicrobial activity of extracts of three different species of genus *Plagichasma* L.et L. from Lonawala, Kas; Satara, and Panhala localities were screened (Table Nos. 9 to 14). They includes *P. articulatum* Kash., *P. appendiculatum* L.et L. and *P. simulensis* Kash. In vitro antibacterial and antifungal efficacy of crude extracts of all species was quantitatively assessed on the basis of inhibition zone and exhibited varying level of inhibitory effect. The inhibition zones were in the range of 7 to 26 mm for most of the tested organisms. Over all, more antibacterial activity accessed by acetone extract of *P. articulatum* Kash. against the *S. aureus* (26 mm) while, low activity against *B. substilis* (7 mm). The *P. articulatum* Kash. acetone extract showed greater antifungal activity against *A. niger* (15 mm) whereas, minimum activity against *F. moniliformae* (7 mm) and *F. oxysporum* (7 mm). A strong fungicidal effect was exerted by extracts of *Plagiochasma* L.et L. against *A. niger* and *F. oxysporum* and bactericidal effect against *S. aureus*. Although remaining all extracts of three species showed varying levels of

moderate activity against all test organisms. But, no antifungal activity against *R. stolonifer*. The results of antifungal activity of *Plagiochasma simulensis* Kash. are clearly indicates that the growth inhibitions against test fungi. Acetone and n-butanol extract of *Plagiochasma simulensis* Kash. was more effective as compare to aqueous and methanol extracts. *F. graminearum* and *A. niger* of n-butanol more sensitive to methanol extracts. On the contrary lowest growth sensitivity was noted for *A. niger* (6 mm) to aqueous extract. It is interesting to note that the *R. stolonifer* was not responding to any extracts. All results were compared with positive control, penicillin (50 µg) where, inhibition of all organisms was altogether absent. Liverworts produce the bioactive substances able to check the growth of pathogenic organisms such as fungi and bacteria.

The present study is an attempt to screen *T. hypophylla* L. extracts against target organisms. The results of antifungal and antibacterial activities were summarized in Table Nos. 15 to 16. Sinhagad region plants and their petroleum ether extracts exhibits greater inhibitory activity against *F. oxysporum* and *E. coli*, whereas, lower activity against *R. stolonifer*, and *S. aureus*, remaining all extracts exhibits variable inhibitory activity. No cidal activity against all tested fungal organisms except *R. stolonifer*. Antibiotic, nyastatin is more sensitive to *F. oxysporum* (13 mm), and amphicillin to *S. aureus* (20 mm).

Organic solvent extracts of *C. tuberosum* Kash. were used for antimicrobial screening and its results were documented in Table Nos. 17 to 18. In the Lonawala regions plant screening, ethanol extract has greater antifungal activity against *A. niger* and antibacterial activity against *S. aureus*; followed by activity of acetone extract against *F. moniliformae*, and *P. aureginosa*. The petroleum ether extract showed 12 mm diameter zone of inhibition against *E. coli*. But, ethanol, petroleum ether and aqueous extracts inactive against *F. moniliformae* and *Rhizopus stolonifer*. According to data, *P. aureginosa* is most sensitive strain with the strongest zone inhibition 13 mm, from

those tested. Amongst these, the gram negative *S. aureus* and *B. substilis* also displayed a variable degree of susceptibility to these extracts. In general found to be more sensitive strain *P. aureginosa*; followed by *E. coli*, *S. aureus* and *B. substilis* with diameter zone of inhibitions 12 mm, 9 mm and 9 mm, respectively. The conventional antibiotic, nystatin are generally more active against *A. niger* and amphicillin against *S. aureus* strain. Extracts from Kas; Satara regions were screened for antimicrobial activities. Among the extracts used, the ethanol extract showed 11 mm diameter zone inhibition against *A. niger*; followed by petroleum ether extract against *F. oxysporum* (9 mm) and water extract exhibiting 8 mm against *A. niger* strain. A maximum zone inhibition 11 mm for ethanol extract whereas, minimum for distilled water extract. The petroleum ether extract showed maximum zone inhibition against *E. coli* (11 mm) and minimum against *S. aureus* 7 mm. Acetone, P. ether and aqueous extract exhibiting no inhibition against *P. aureginosa* strain. The chemical constituents of particular plant species varies according to geographical area and season therefore, variation in antibiotic activities is possible. The nystatin more active against *F. moniliformae* and amphicillin against *P. aureginosa* strain which used to compare antibiotic activity.

Riccia discolar L. extracts results were summarized in Table Nos. 19 to 20. Plant material from Purandar used to screen antimicrobial properties. Here, ethanol extract exhibits greater antifungal activity against *A. niger* (8.6 mm) and acetone extract against *S. aureus* (14.5 mm), whereas lower activity against *P. aeruginosa* (7 mm). Antifungal and antibacterial activities of *R. fluitans* L. extracts against target organisms were depicted in Table Nos. 21 to 22. Acetone extract exhibits greater antifungal activity against *F. oxysporum* (13 mm), whereas lower activity against *R. stolonifer* (7 mm). Ethanol extract exhibits more antibacterial activity against *S. aureus* (21 mm). The antibiotic nyastatin used as positive control showed greater inhibitory activity against *A. niger* (17 mm), and amphicillin against *E. coli* (23 mm).

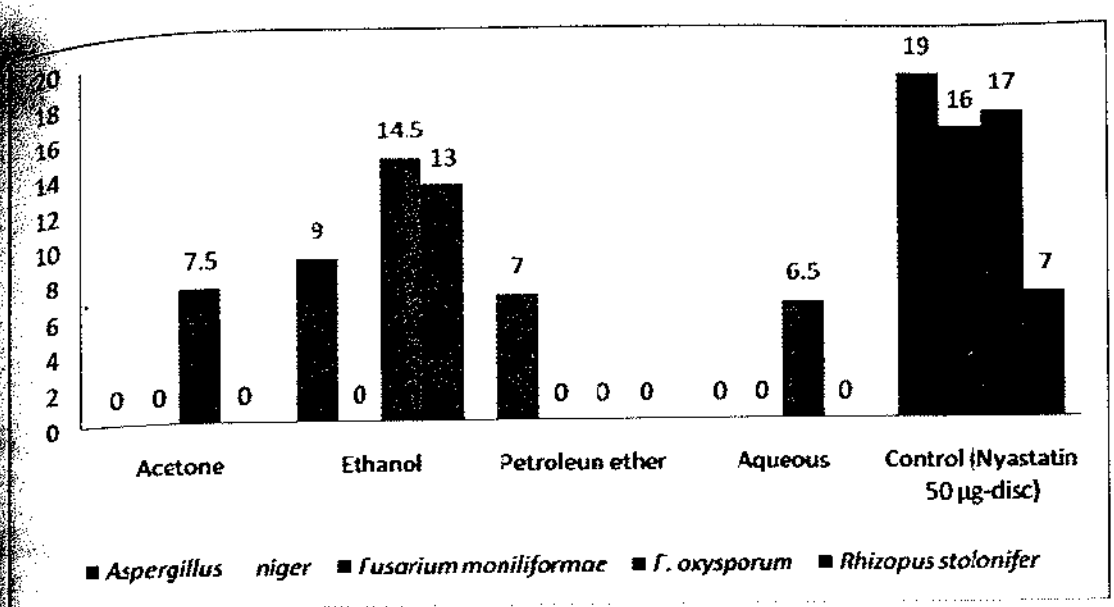
Determination of antimicrobial activity by 'Disc diffusion assay' method.

5 -E, Table No. 1 : Antifungal screening of *Fossombronia indica* St. extracts against test organisms.

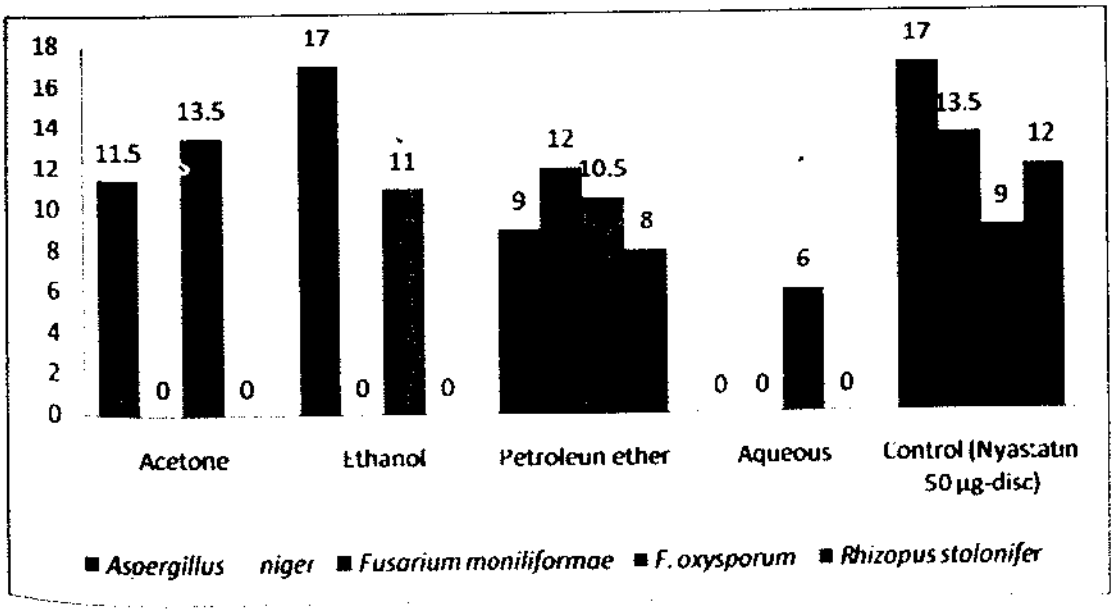
	F. indica St. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Purandar	Acetone	NI	NI	7.5	NI
	Ethanol	9.0	NI	14.5	13.0
	Petroleum ether	7.0	NI	NI	NI
	Aqueous	NI	NI	6.5	NI
	Control, (Nyastatin 50 μg^{-disc})	19.0	16.0	17.0	NI
Panhala	Acetone	11.5	NI	13.5	NI
	Ethanol	17.0	NI	11.0	NI
	Petroleum ether	9.0	12.0	10.5	8.0
	Aqueous	NI	NI	6.0	NI
	Control, (Nyastatin 50 μg^{-disc})	17.0	13.5	9.0	12.0

NI : No Inhibition

1. Antifungal activity of *Fossombronia indica* St. extracts against test organisms at Purandar.



2. Antifungal activity of *Fossombronia indica* St. extracts against test organisms at Panhala.



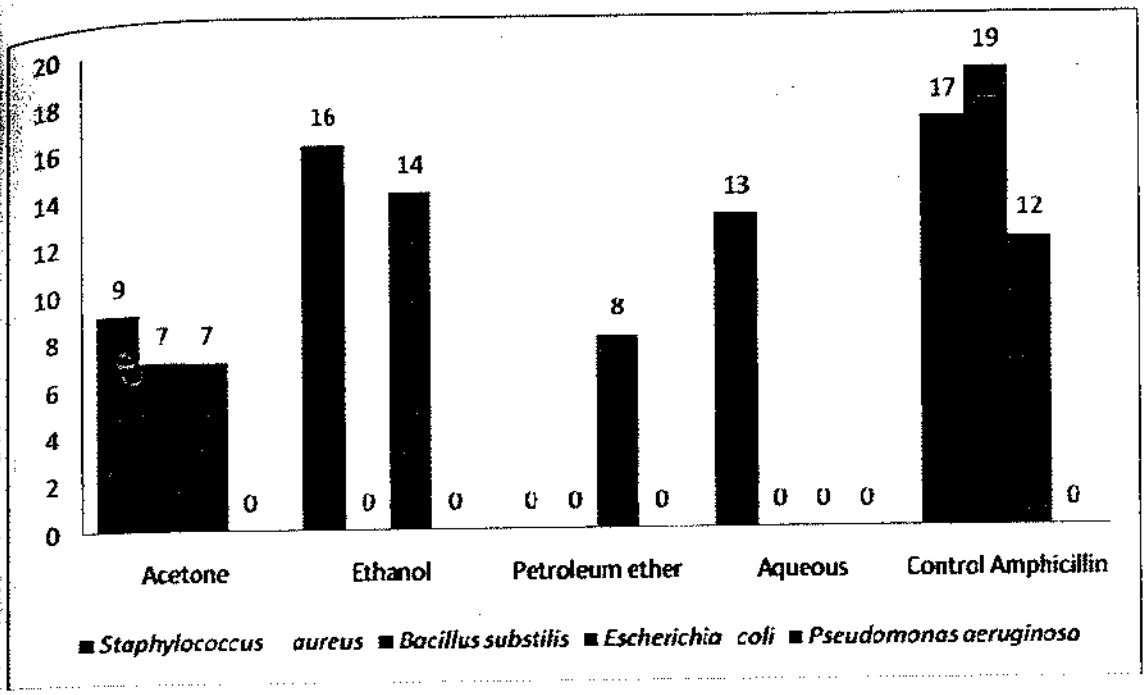
Y- axis : Values of inhibition zone (mm).

Table No. 2 Antibacterial screening of *Fossombronia indica* St. extracts against test organisms.

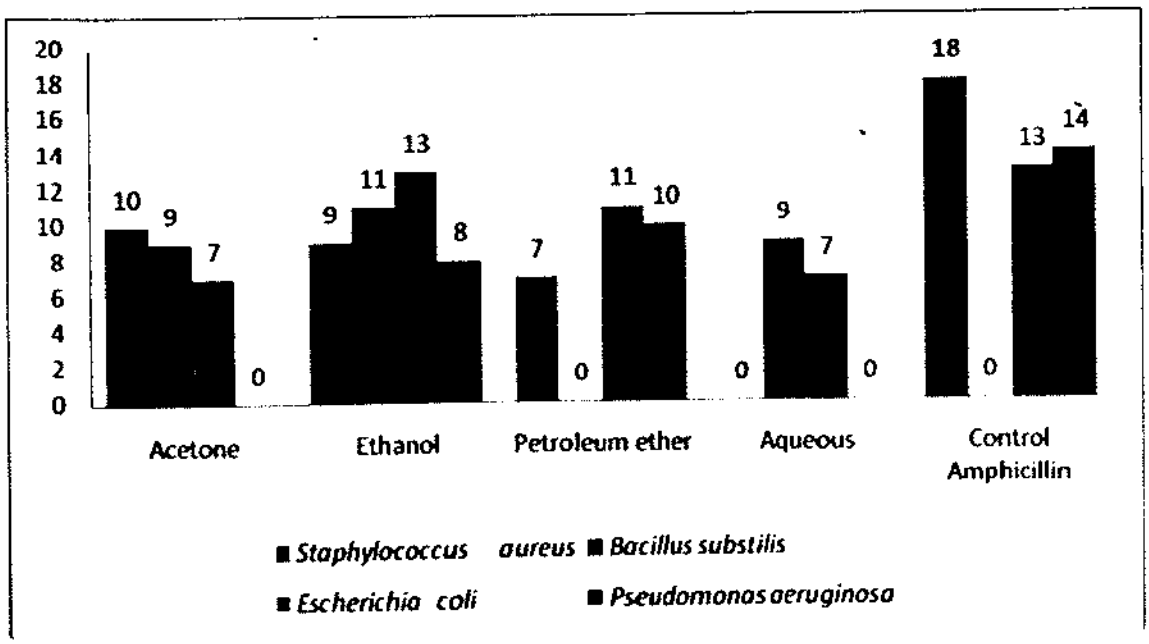
	F. indica St. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Purandar	Acetone	9.0	7.0	7.0	NI
	Ethanol	16.0	NI	14.0	NI
	Petroleum ether	NI	NI	8.0	NI
	Aqueous	13.0	NI	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	17.0	19.0	12.0	NI
Panhala	Acetone	10.0	9.0	7.0	NI
	Ethanol	9.0	11.0	13.0	8.0
	Petroleum ether	7.0	NI	11.0	10.0
	Aqueous	NI	9.0	7.0	NI
	Control, (Amphicillin 25 μg^{-disc})	18.0	NI	13.0	14.0

NI : No Inhibition

3. Antibacterial activity of *Fossombronia indica* St. extracts against test organisms at Purandar.



4. Antibacterial activity of *Fossombronia indica* St. extracts against test organisms at Panhala.



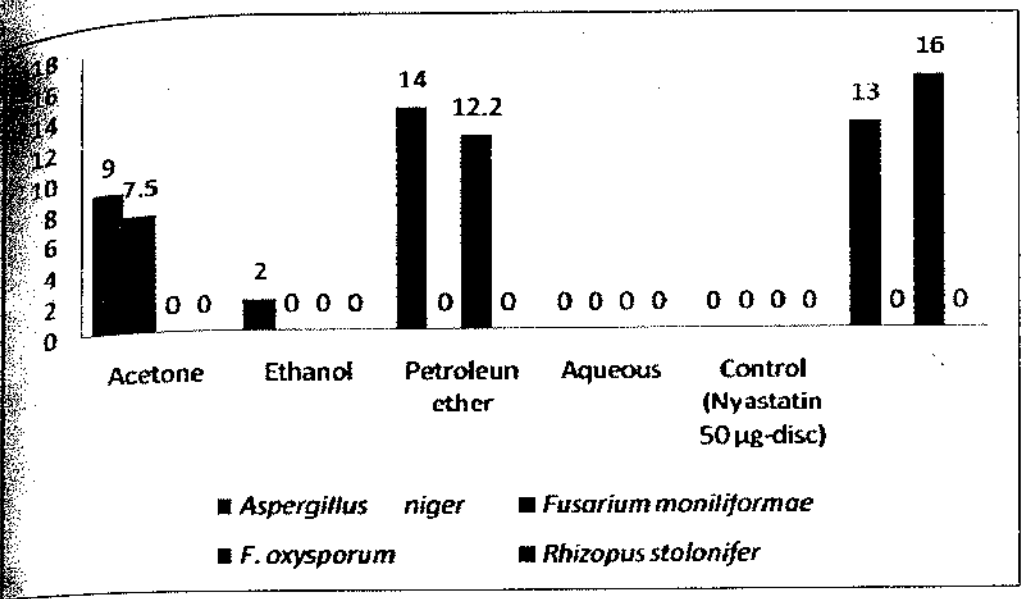
Y- axis : Values of inhibition zone (mm).

Table No. 3 Antifungal screening of *Sewardiella tuberifera* Kash. extracts against test organisms.

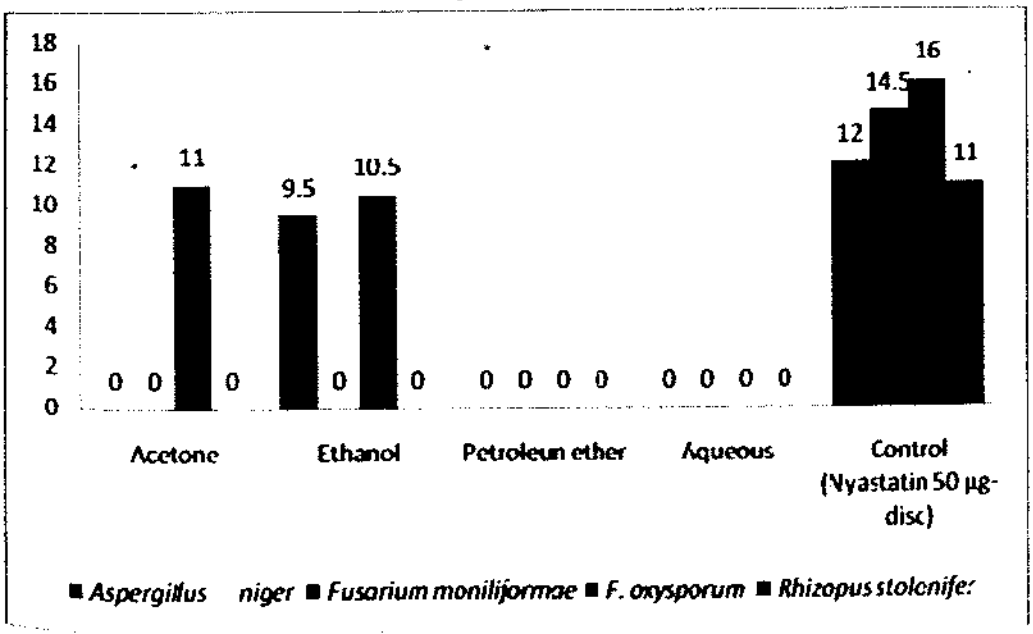
	S. tuberifera Kash. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Purandar	Acetone	9.2	7.5	NI	NI
	Ethanol	14.0	NI	NI	NI
	Petroleum ether	NI	NI	12.2	NI
	Aqueous	NI	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	13.0	NI	16.0	NI
Sinhagad	Acetone	NI	NI	11.0	NI
	Ethanol	9.5	NI	10.5	NI
	Petroleum ether	NI	NI	NI	NI
	Aqueous	NI	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	12.0	14.5	16.0	11

NI : No Inhibition

5. Antifungal activity of *Sewardiella tuberifera* Kash. extracts against test organisms at Purandar.



6. Antifungal activity of *Sewardiella tuberifera* Kash. extracts against test organisms at Sinhagad.



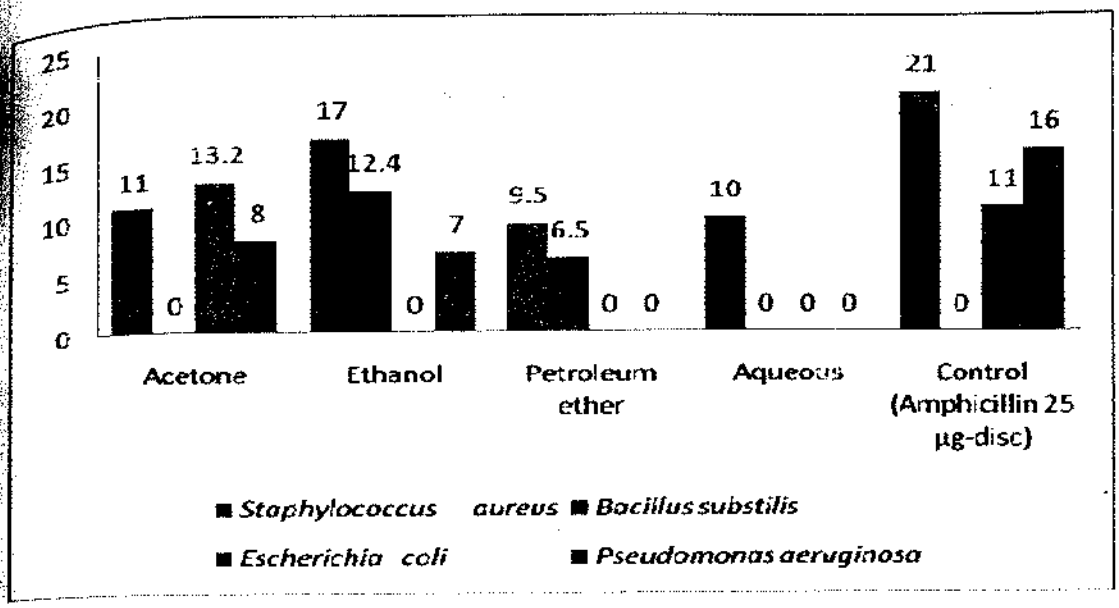
Y- axis : Values of inhibition zone (mm)

Table No. 4 Antibacterial screening of *Sewardiella tuberifera* Kash. extracts against test organisms.

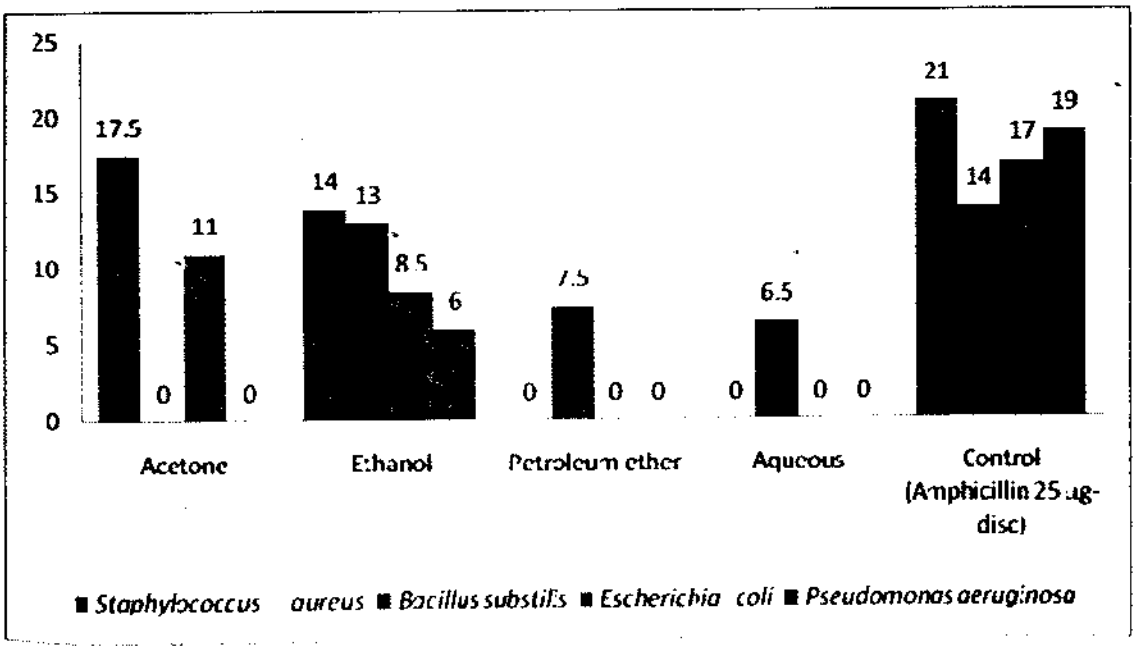
	S. tuberifera Kash. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Purandar	Acetone	11.0	NI	13.2	8.0
	Ethanol	17.0	12.4	NI	7.0
	Petroleum ether	9.5	6.5	NI	NI
	Aqueous	10.0	NI	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	21.0	NI	11.0	16.0
Sinhagad	Acetone	17.5	NI	11.0	NI
	Ethanol	14.0	13.0	8.5	6.0
	Petroleum ether	NI	7.5	NI	NI
	Aqueous	NI	6.5	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	21.0	14.0	17.0	19.0

NI : No Inhibition

7. Antibacterial activity of *Sewardiella tuberifera* Kash. extracts against test organisms at Purandar.



8. Antibacterial activity of *Sewardiella tuberifera* Kash. extracts against test organisms at Sinhagad.



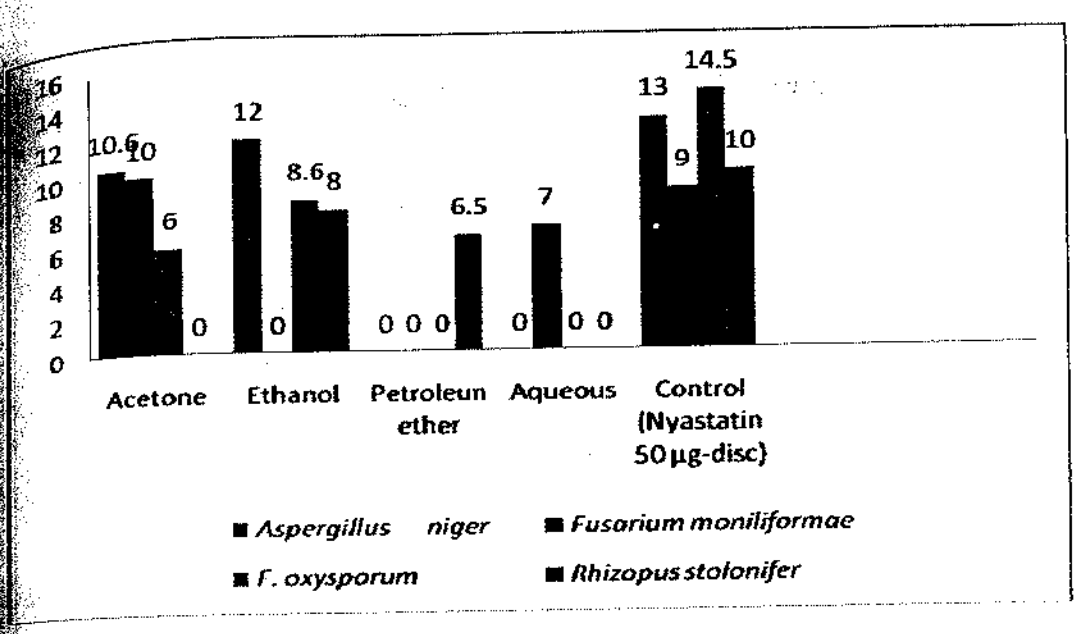
Y- axis : Values of inhibition zone (mm).

Table No. 5 Antifungal screening of *Exormotheca tuberifera* L. extracts against test organisms.

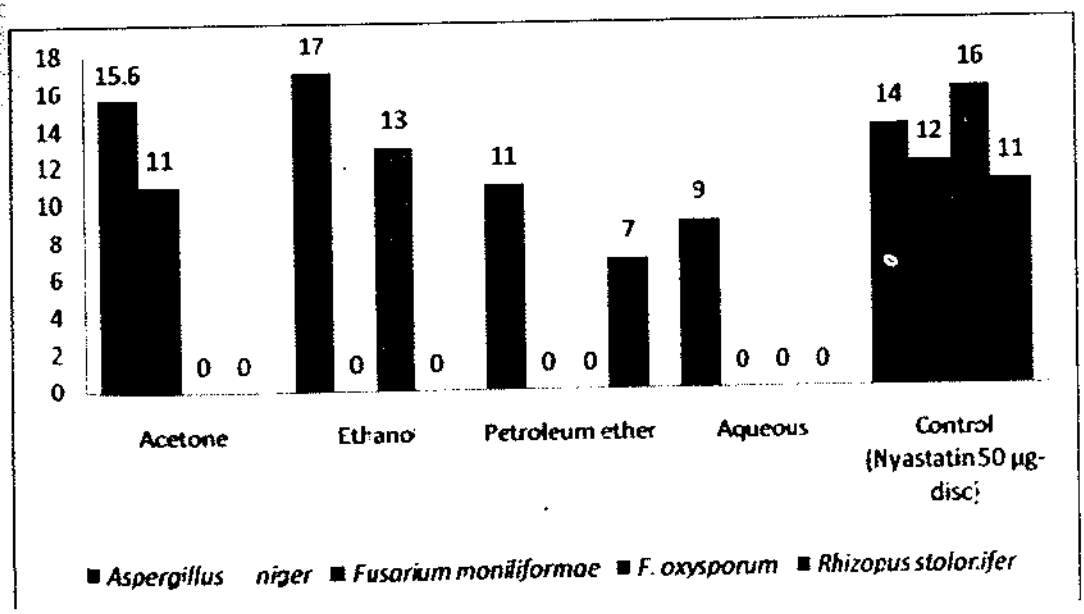
	E. tuberifera L. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Purandar	Acetone	10.6	NI	6.0	NI
	Ethanol	12.0	NI	8.6	8.0
	Petroleum ether	NI	NI	NI	6.5
	Aqueous	NI	7.0	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	13.0	9.0	14.5	10.0
Khandala	Acetone	15.6	11.0	NI	NI
	Ethanol	17.0	NI	13.0	NI
	Petroleum ether	11.0	NI	NI	7.0
	Aqueous	9.0	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	14.0	12.0	16.0	11.0

NI : No Inhibition

9. Antifungal activity of *Exormotheca tuberifera* L. extracts against test organisms at Purandar.



10. Antifungal activity of *Exormotheca tuberifera* L. extracts against test organisms at Khandala.



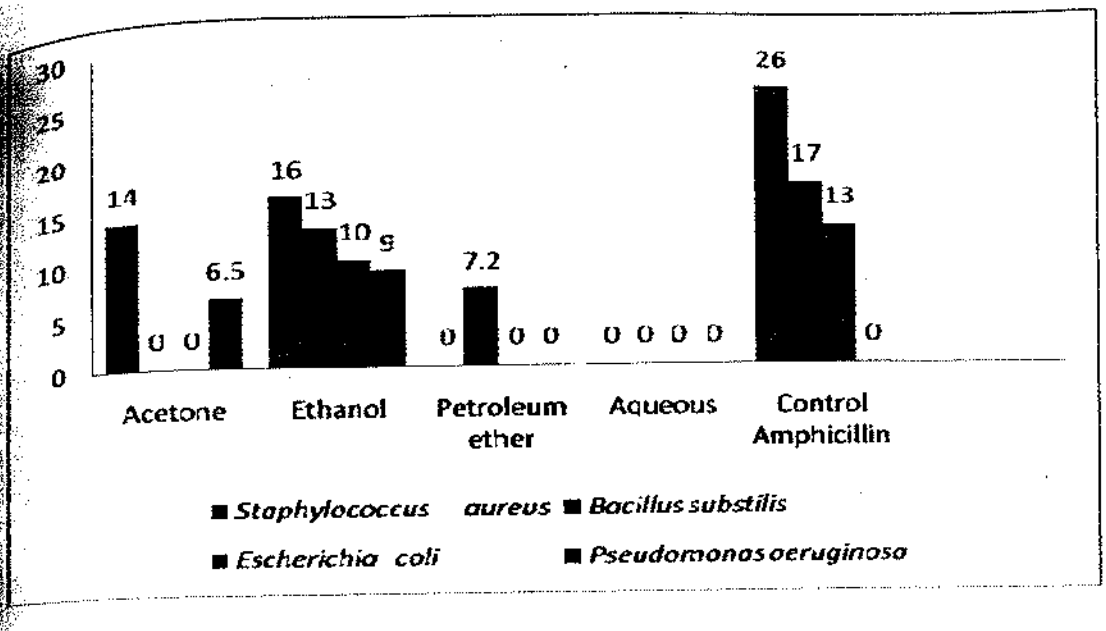
Y- axis : Values of inhibition zone (mm).

Table No. 6 Antibacterial screening of *Exormotheca tuberifera* L. extracts against test organisms.

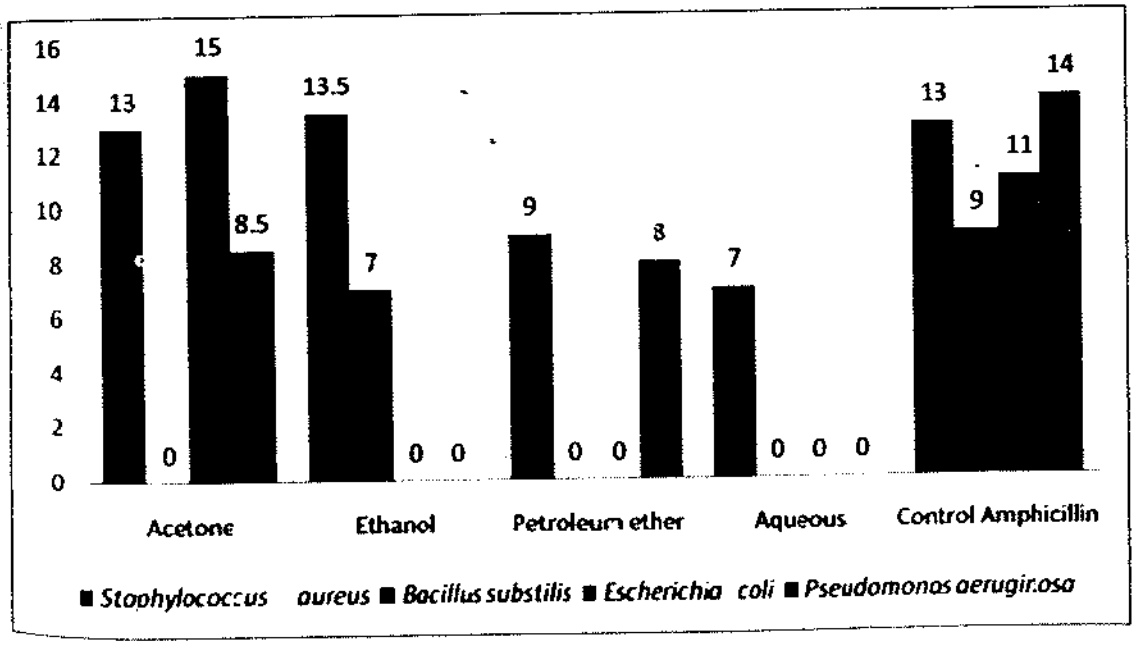
	E. tuberifera L. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Purandar	Acetone	14.0	NI	NI	6.5
	Ethanol	16.0	13.0	10.0	9.0
	Petroleum ether	NI	7.2	NI	NI
	Aqueous	NI	NI	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	26.0	17.0	13.0	NI
Khandala	Acetone	13.0	NI	15.0	8.5
	Ethanol	13.5	7.0	NI	NI
	Petroleum ether	9.0	NI	NI	8.0
	Aqueous	7.0	NI	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	13.0	9.0	11.0	14.0

NI : No Inhibition

11. Antibacterial activity of *Exormotheca tuberifera* L. extracts against test organisms at Purandar.



12. Antibacterial activity of *Exormotheca tuberifera* L. extracts against test organisms at Khandala.



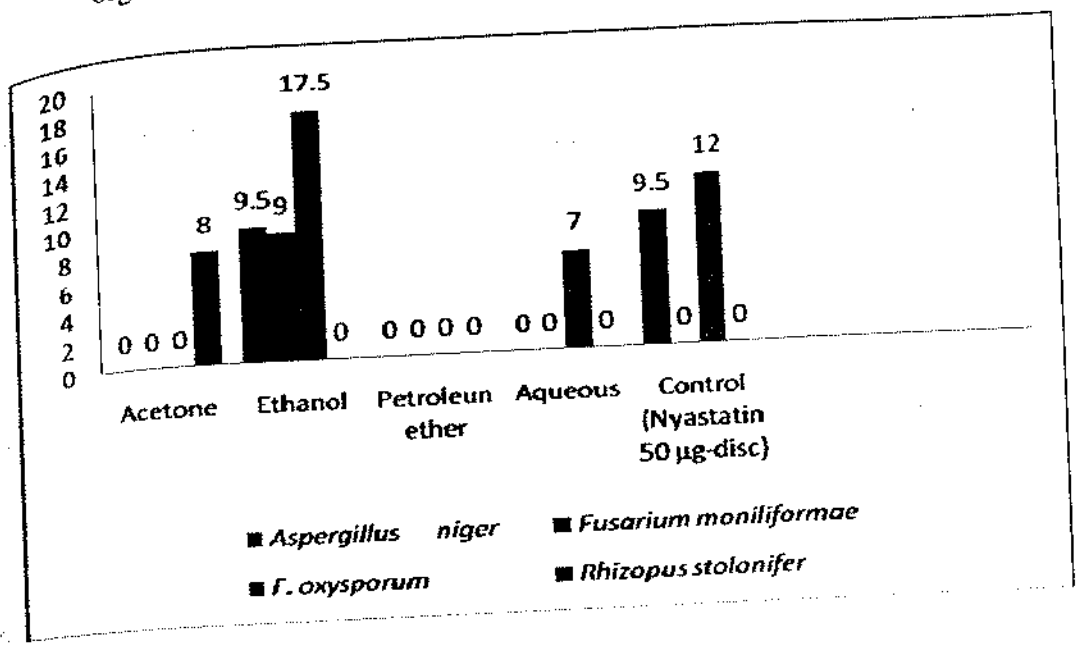
Y- axis : Values of inhibition zone (mm).

Table No. 7 Antifungal screening of *Asterella angusta* St. extracts against test organisms.

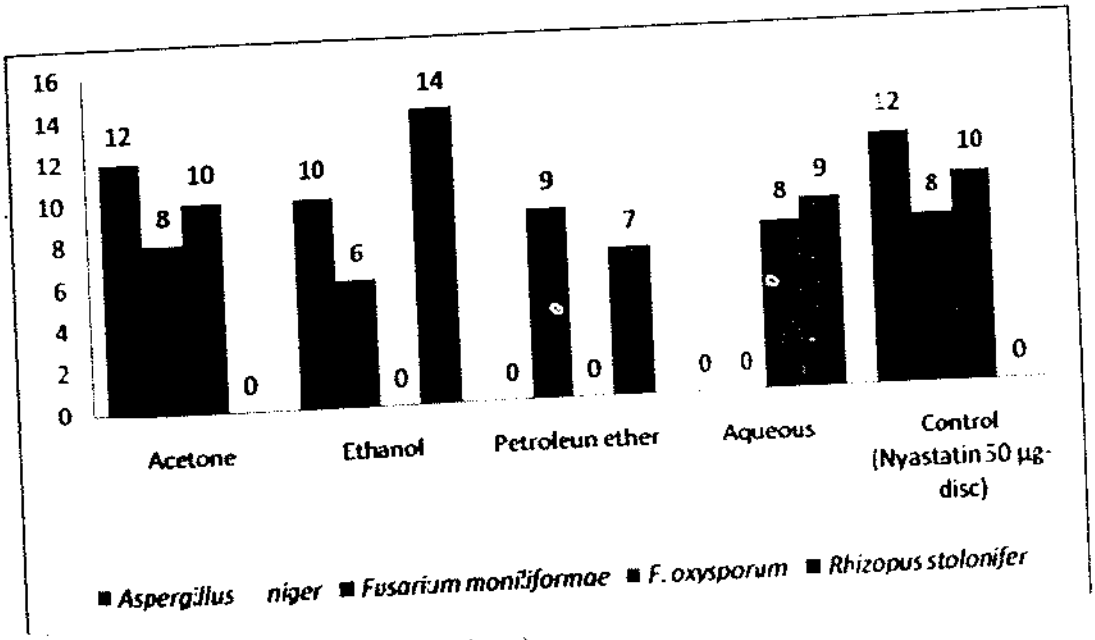
	A. angusta St. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Rajgad	Acetone	NI	NI	NI	8.0
	Ethanol	9.5	9.0	17.5	NI
	Petroleum ether	NI	NI	NI	NI
	Aqueous	NI	NI	7.0	NI
	Control, (Nyastatin 50 μg^{-disc})	9.5	NI	12.0	NI
Lonawala	Acetone	12.0	8.0	10	NI
	Ethanol	10.0	6.0	NI	14
	Petroleum ether	NI	9.0	NI	7.0
	Aqueous	NI	NI	8.0	9.0
	Control, (Nyastatin 50 μg^{-disc})	12.0	8.0	10	NI

NI : No Inhibition

13. Antifungal activity of *Asterella angusta* St. extracts against test organisms at Rajgad.



14. Antifungal activity of *Asterella angusta* St. extracts against test organisms Lonawala.



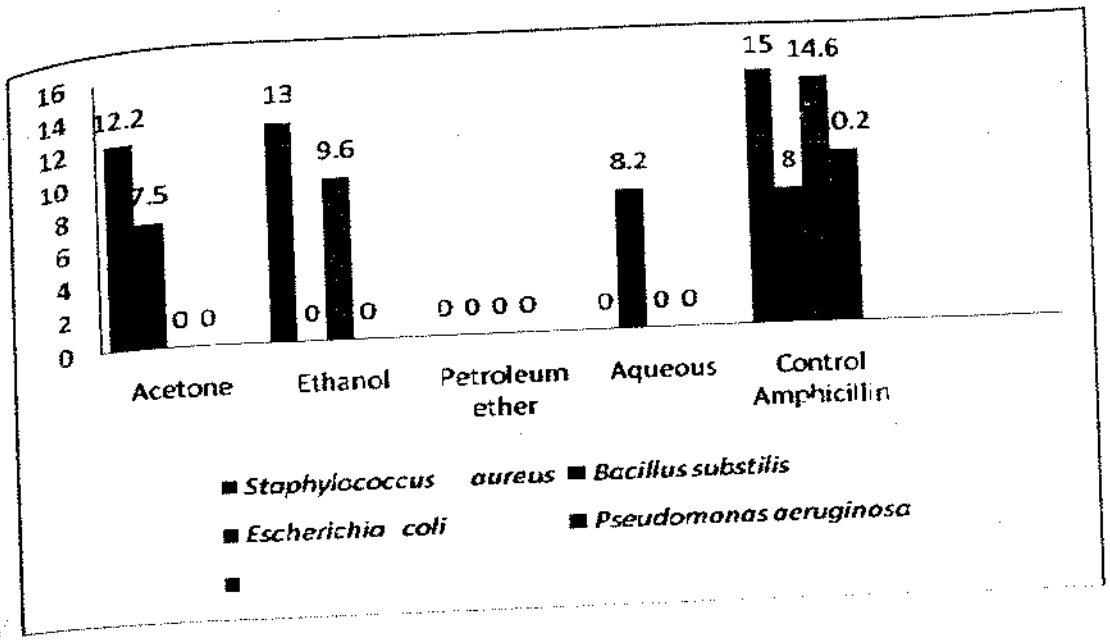
Y- axis : Values of inhibition zone (mm).

Table No. 8 Antibacterial screening of *Asterella angusta* St. extracts against test organisms.

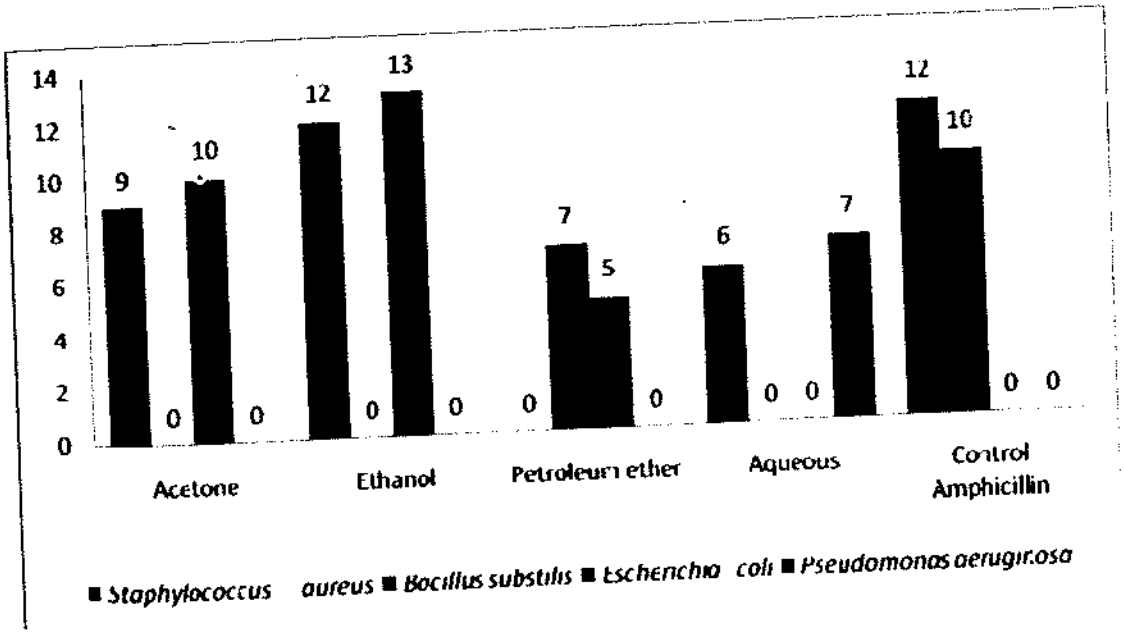
	A. angusta St. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Rajgad	Acetone	12.2	7.5	NI	NI
	Ethanol	13.0	NI	9.6	NI
	Petroleum ether	NI	NI	NI	NI
	Aqueous	NI	8.2	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	15.0	8.0	14.6	10.2
Lonawala	Acetone	9.0	NI	10.0	NI
	Ethanol	12.0	NI	13.0	NI
	Petroleum ether	NI	7.0	5.0	NI
	Aqueous	6.0	NI	NI	7.0
	Control, (Amphicillin 25 μg^{-disc})	12.0	10.0	NI	NI

NI : No Inhibition

15. Antibacterial activity of *Asterella angusta* St. extracts against test organisms at Rajgad.



16. Antibacterial activity of *Asterella angusta* St. extracts against test organisms at Lonawala.



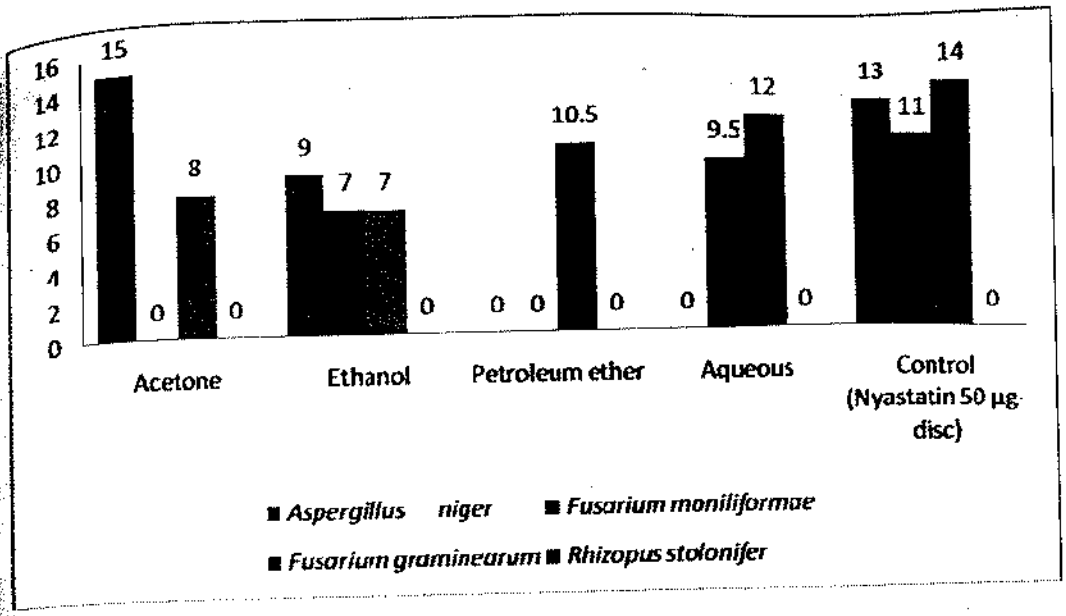
Y- axis : Values of inhibition zone (mm).

Table No. 9 Antifungal screening of *Plagiochasma articulatum* Kash. extracts against test organisms.

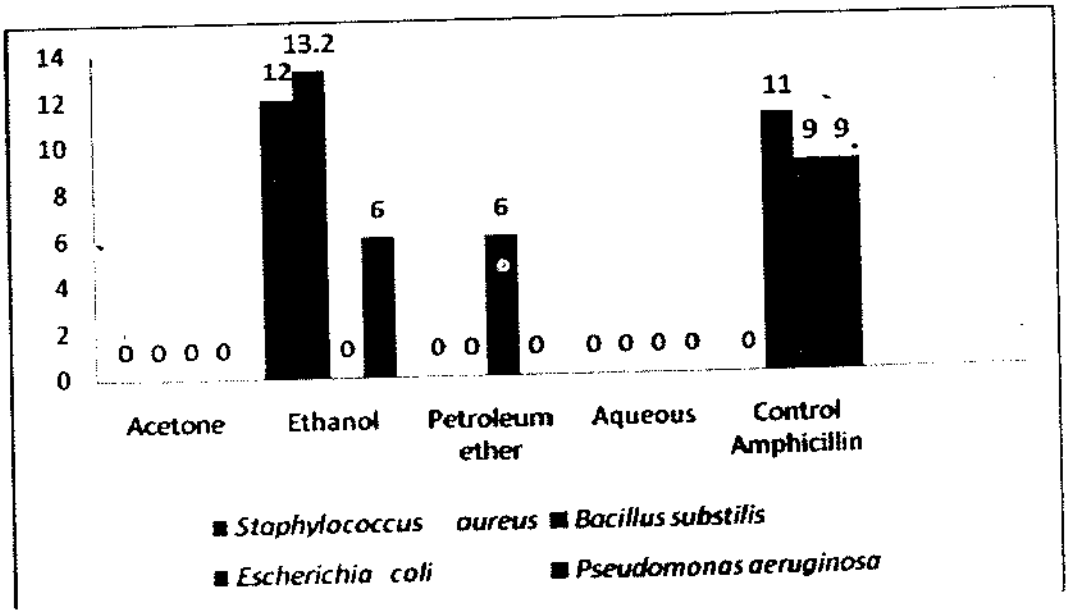
	P. articulatum Kash. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Purandar	Acetone	15.0	NI	8.0	NI
	Ethanol	9.0	NI	7.0	NI
	Petroleum ether	NI	NI	10.5	NI
	Aqueous	NI	9.5	12.0	NI
	Control, (Nyastatin 50 μg^{-disc})	13.0	11.0	14.0	NI
Raigad	Acetone	NI	NI	NI	NI
	Ethanol	12.0	13.2	NI	6.0
	Petroleum ether	NI	NI	6.0	NI
	Aqueous	NI	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	NI	11.0	9.0	9.0

NI : No Inhibition

17. Antifungal activity of *Plagiochasma articulatum* Kash. extracts against test organisms at Purandar.



18. Antifungal activity of *Plagiochasma articulatum* Kash. extracts against test organisms Raigad.



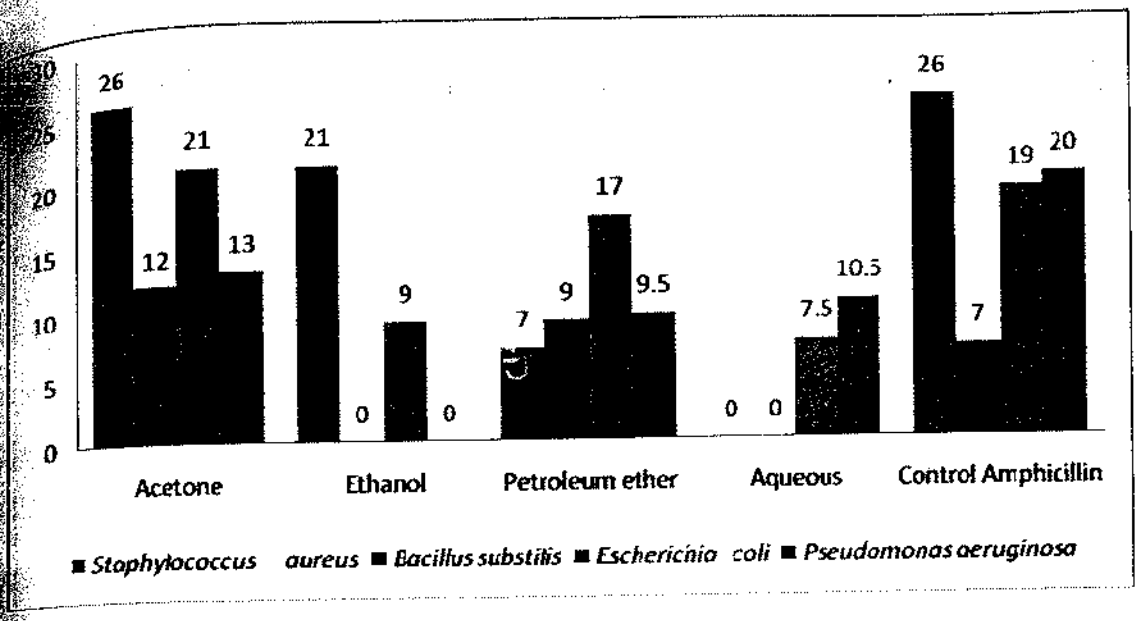
Y- axis : Values of inhibition zone (mm).

Table No. 10 Antibacterial screening of *P. articulatum* Kash. extracts against test organisms.

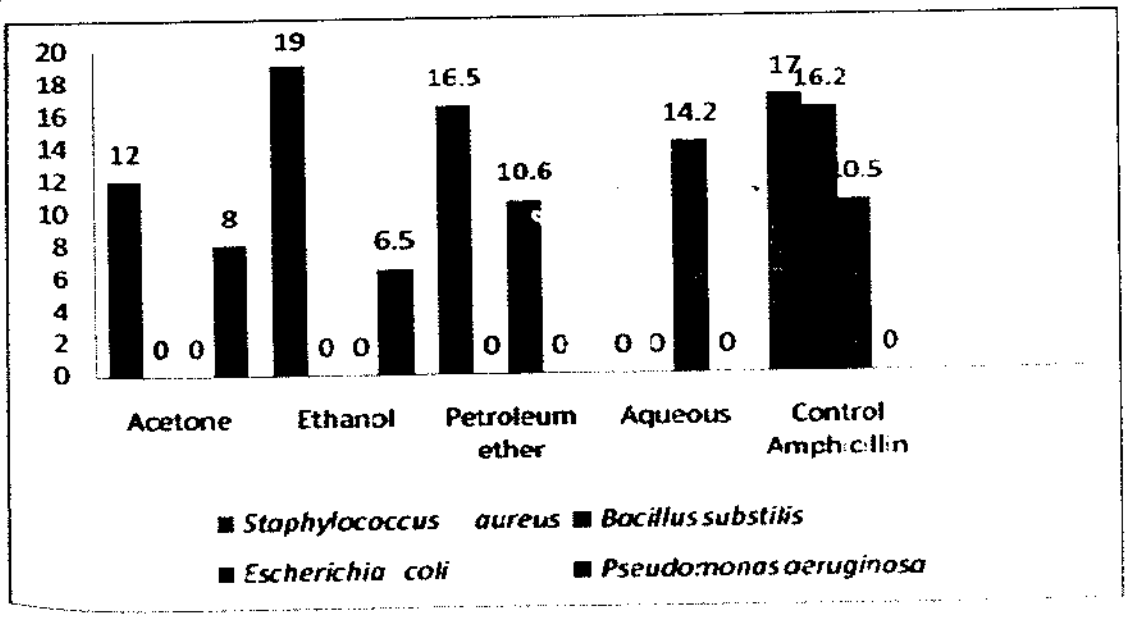
	P. articulatum Kash. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Purandar	Acetone	26.0	12.0	21.0	13.0
	Ethanol	21.0	NI	9.0	NI
	Petroleum ether	7.0	9.0	17.0	9.5
	Aqueous	NI	NI	7.5	10.5
	Control, (Amphicillin 25 μg^{-disc})	26.0	7.0	19.0	20.0
Raigad	Acetone	12.0	NI	NI	8.0
	Ethanol	19.0	NI	NI	6.5
	Petroleum ether	16.5	NI	10.6	NI
	Aqueous	NI	NI	14.2	NI
	Control, (Amphicillin 25 μg^{-disc})	17.0	16.2	10.5	NI

NI : No Inhibition

19. Antibacterial activity of *P. articulatum* Kash. extracts against test organisms at Purandar.



20. Antibacterial activity of *P. articulatum* Kash. extracts against test organisms at Raigad.



Y- axis : Values of inhibition zone (mm).

Table No. 11 Antifungal screening of *P. appendiculatum* L. extracts against test organisms.

	P. appendiculatum L.et L. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Kas;Satara	Acetone	10.0	NI	9.0	9.0
	Methanol	14.5	8.0	13.0	10.0
	n- butanol	NI	12.0	11.0	NI
	Aqueous	11.0	7.0	9.0	NI
	Control, (Nyastatin 50 μg^{-disc})	17.0	11.0	11.0	NI
Khandala	Acetone	8.2	NI	NI	NI
	Ethanol	NI	12.8	NI	9.2
	Petroleum ether	NI	NI	NI	7.0
	Aqueous	8.2	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	10.0	19.0	16.6	12.0

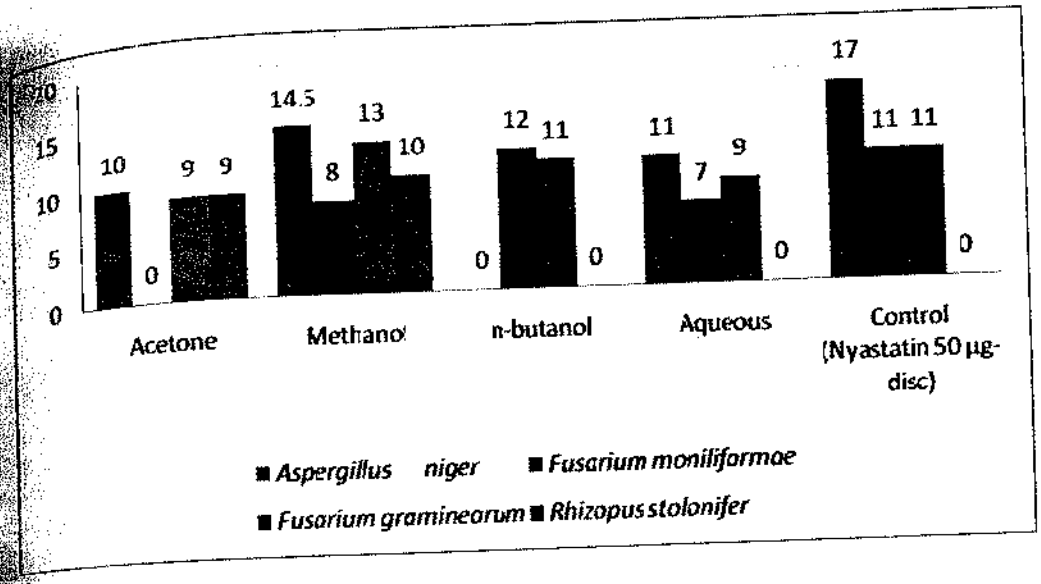
NI : No Inhibition

Table No. 11 Antifungal screening of *P. appendiculatum* L. extracts against test organisms.

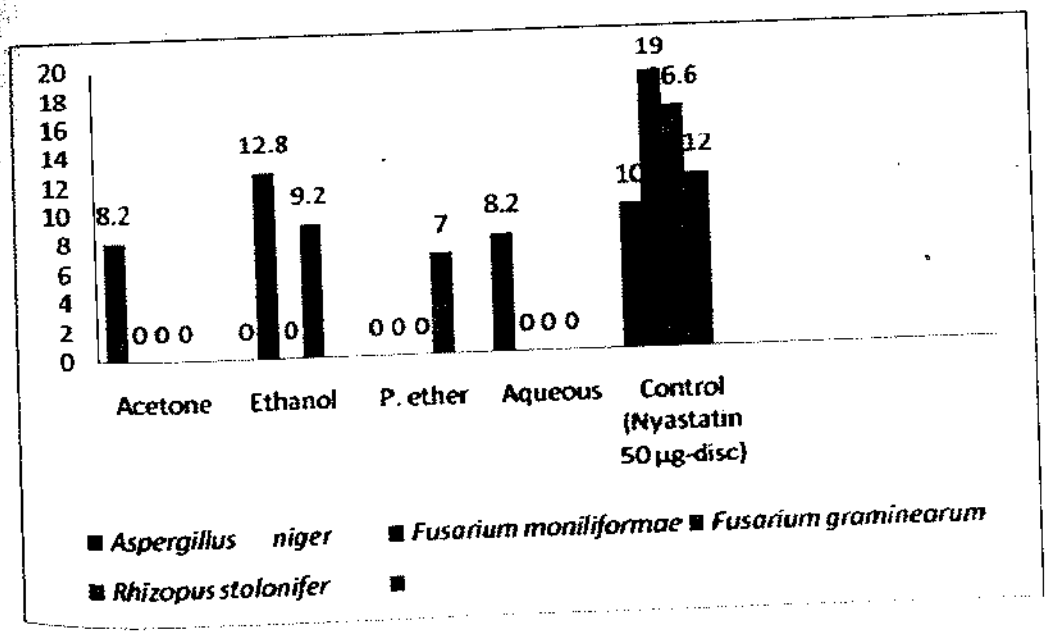
	P. appendiculatum L.et L. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Kas;Satara	Acetone	10.0	NI	9.0	9.0
	Methanol	14.5	8.0	13.0	10.0
	n- butanol	NI	12.0	11.0	NI
	Aqueous	11.0	7.0	9.0	NI
	Control, (Nyastatin 50 μg^{-disc})	17.0	11.0	11.0	NI
Khandala	Acetone	8.2	NI	NI	NI
	Ethanol	NI	12.8	NI	9.2
	Petroleum ether	NI	NI	NI	7.0
	Aqueous	8.2	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	10.0	19.0	16.6	12.0

NI : No Inhibition

21. Antifungal activity of *P. appendiculatum* L. extracts against test organisms at Kas;Satara.



22. Antifungal activity of *P. appendiculatum* L. extracts against test organisms at Khandala.



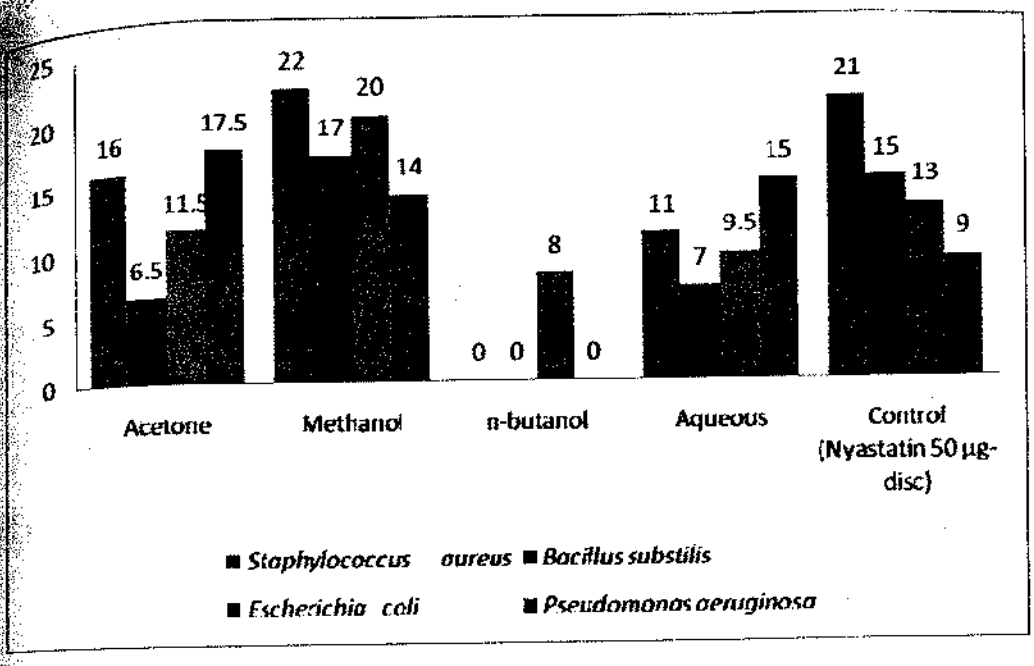
Y- axis : Values of inhibition zone (mm).

Table No. 12 Antibacterial screening of *P. appendiculatum* L. extracts against test organisms.

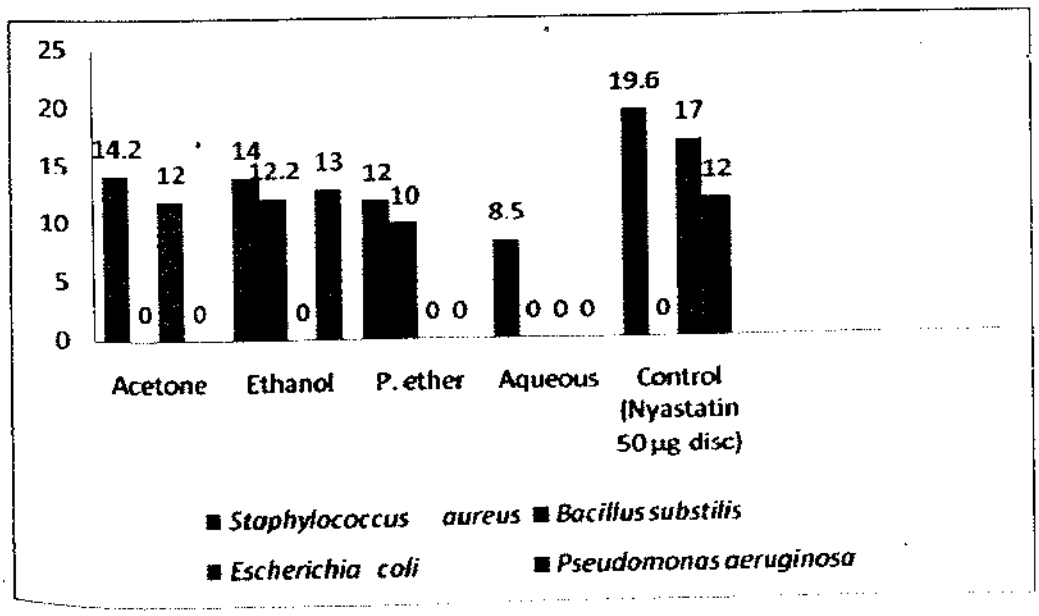
	P. appendiculatum L.et L. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Kas;Satara	Acetone	16.0	6.5	11.5	17.5
	Methanol	22.0	17.0	20.0	14.0
	n- butanol	NI	NI	8.0	NI
	Aqueous	11.0	7.0	9.5	15.0
	Control, (Amphicillin 25 μg^{-disc})	21.0	15.0	13.0	9.0
Khandala	Acetone	14.2	NI	12.0	NI
	Ethanol	14.0	12.2	NI	13.0
	Petroleum ether	12.0	10.0	NI	NI
	Aqueous	8.5	NI	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	19.6	NI	17.0	12.0

NI : No Inhibition

23. Antibacterial activity of *P. appendiculatum* L. extracts against test organisms at Kas; Satara.



24. Antibacterial activity of *P. appendiculatum* L. extracts against test organisms at Khandala.



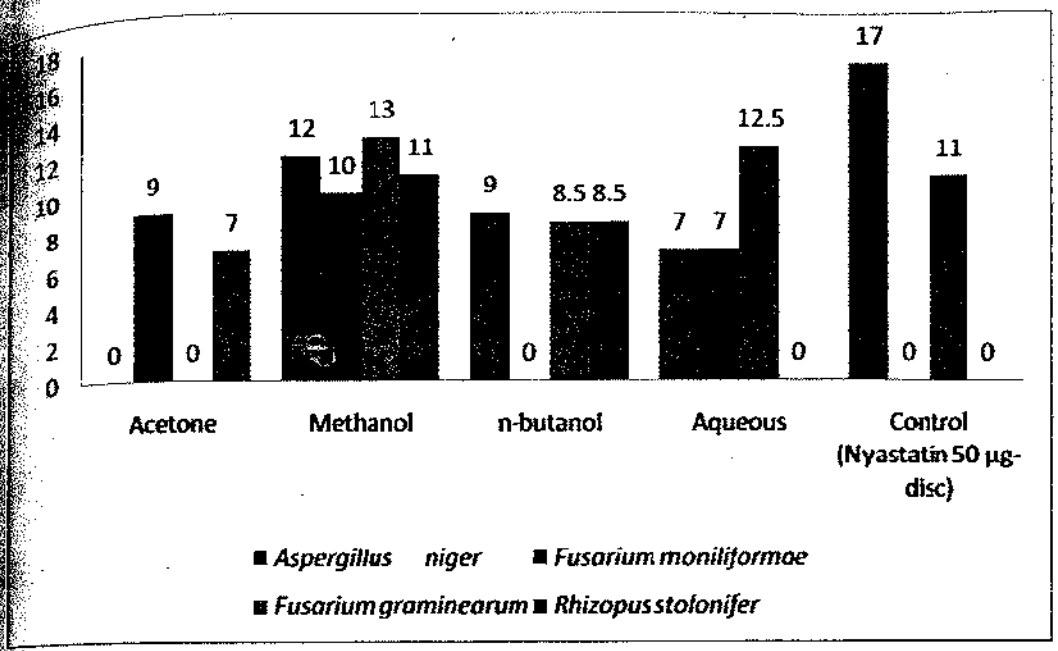
Y- axis : Values of inhibition zone (mm).

Table No. 13 Antifungal screening of *P. simulensis* Kash. extracts against test organisms.

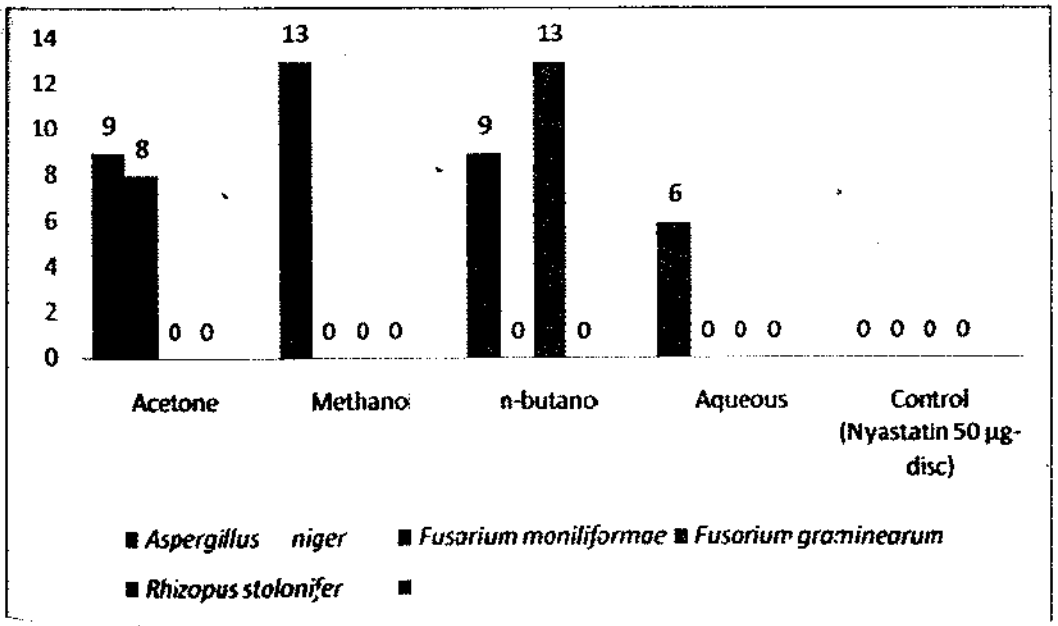
	P. simulensis Kash. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Panhala	Acetone	NI	9.0	NI	7.0
	Ethanol	12.0	10.0	13.0	11.0
	Petroleum ether	9.0	NI	6.5	8.5
	Aqueous	7.0	7.0	12.5	NI
	Control (Nyastatin 50 μg^{-disc})	17.0	NI	11.0	NI
Sinhagad	Acetone	9.0	8.0	NI	NI
	Ethanol	13.0	NI	NI	NI
	Petroleum ether	9.0	NI	13.0	NI
	Aqueous	6.0	NI	NI	NI
	Control (Nyastatin 50 μg^{-disc})	NI	NI	NI	NI

NI : No Inhibition

25. Antifungal activity of *P. simulensis* Kash. extracts against test organisms at Panhala.



26. Antifungal activity of *P. simulensis* Kash. extracts against test organisms at Sinhagad.



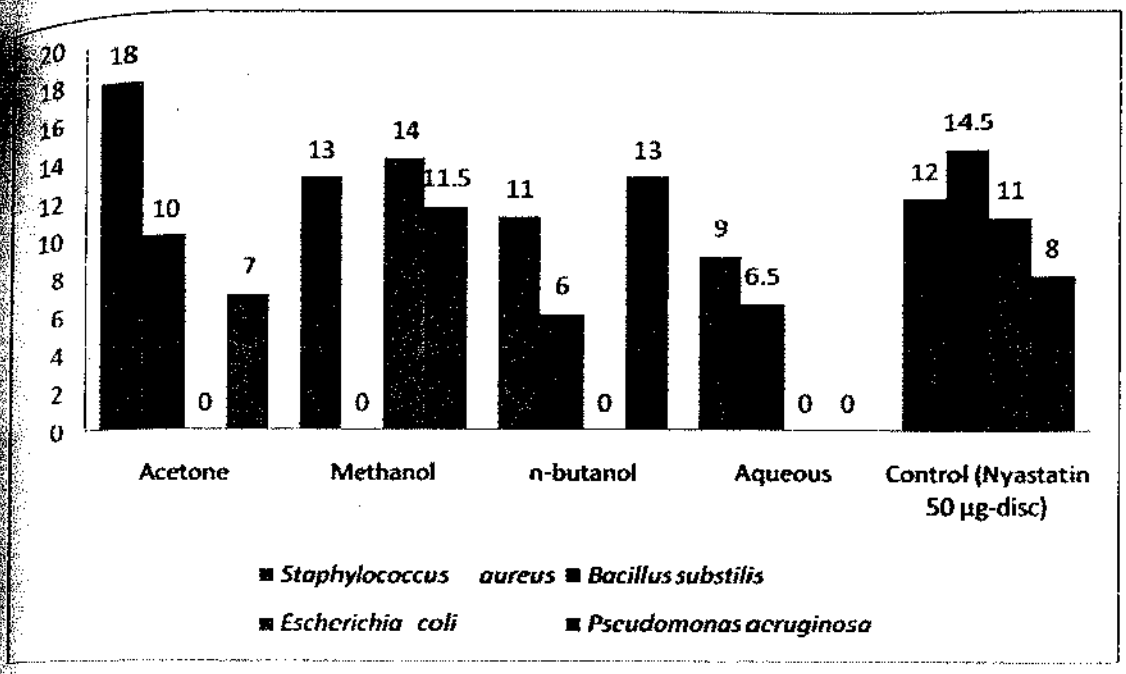
Y- axis : Values of inhibition zone (mm).

Table No. 14 Antibacterial screening of *P. simulensis* Kash. extracts against test organisms.

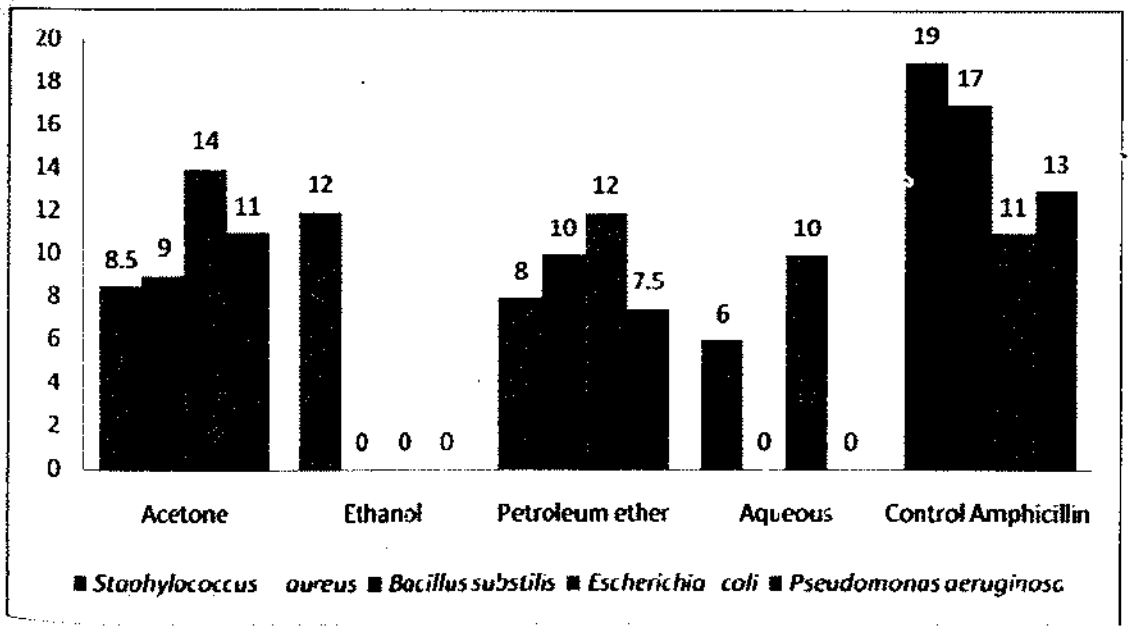
	P. simulensis Kash. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Panhala	Acetone	18.0	10.0	NI	7.0
	Ethanol	13.0	NI	14.0	11.5
	Petroleum ether	11.0	6.0	NI	13.0
	Aqueous	9.0	6.5	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	12.0	14.5	11.0	8.0
Sinhagad	Acetone	8.5	9.0	14.0	11.0
	Ethanol	12.0	NI	NI	NI
	Petroleum ether	8.0	10.0	12.0	7.5
	Aqueous	6.0	NI	10.0	NI
	Control, (Amphicillin 25 μg^{-disc})	19.0	17.0	11.0	13.0

NI : No Inhibition

27. Antibacterial activity of *P. simulensis* Kash. extracts against test organisms at Panhala.



28. Antibacterial activity of *P. simulensis* Kash. extracts against test organisms at Sinhagad.



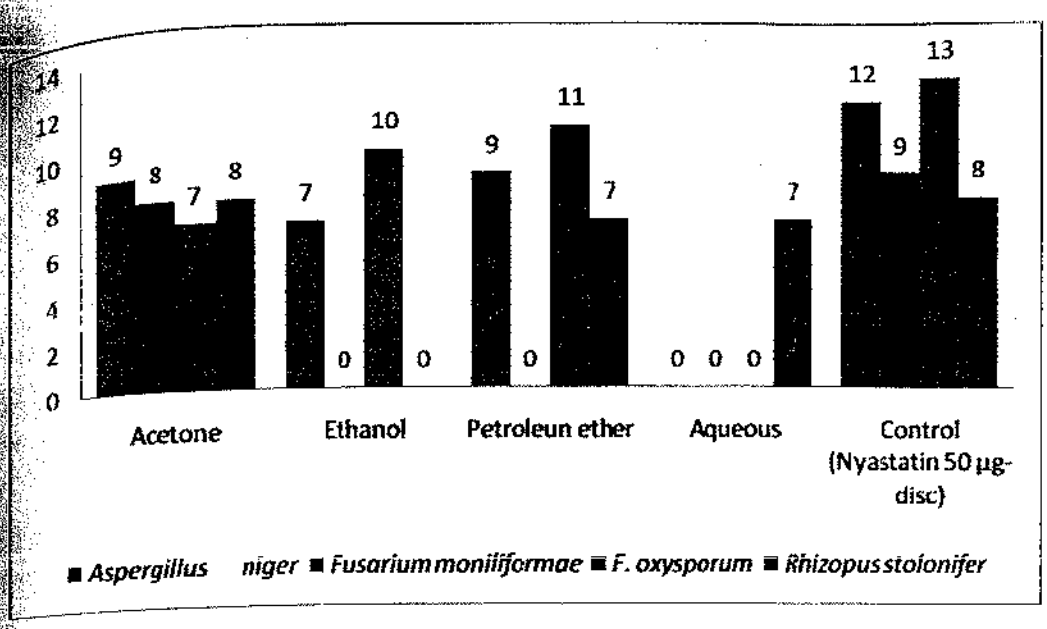
Y- axis : Values of inhibition zone (mm).

Table No.15 Antifungal screening of *Targionia hypophylla* L. extracts against test organisms.

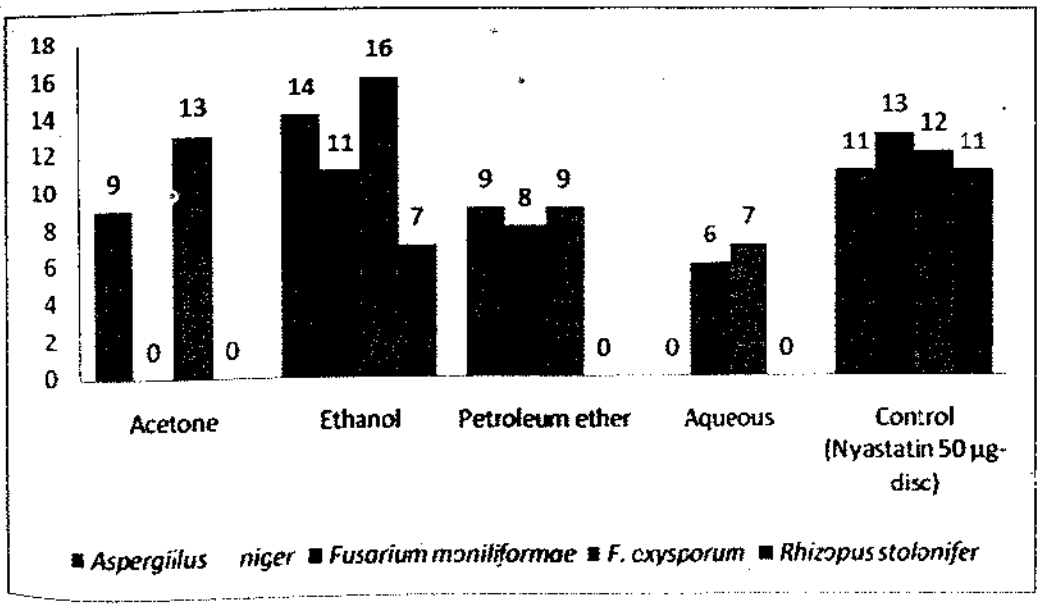
	T. hypophylla L. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Sinhagad	Acetone	9.0	8.0	7.0	8.0
	Ethanol	7.0	NI	10.0	NI
	Petroleum ether	9.0	NI	11.0	7.0
	Aqueous	NI	NI	NI	7.0
	Control, (Nyastatin 50 μg^{-disc})	12.0	9.0	13.0	8.0
Rayreshwar	Acetone	9.0	NI	13.0	NI
	Ethanol	14.0	11.0	16.0	7.0
	Petroleum ether	9.0	8.0	9.0	NI
	Aqueous	NI	6.0	7.0	NI
	Control, (Nyastatin50 μg^{-disc})	11.0	13.0	12.0	11.0

NI : No Inhibition

29. Antifungal activity of *Targionia hypophylla* L. extracts against test organisms at Sinhagad.



30. Antifungal activity of *Targionia hypophylla* L. extracts against test organisms at Rayreshwar.



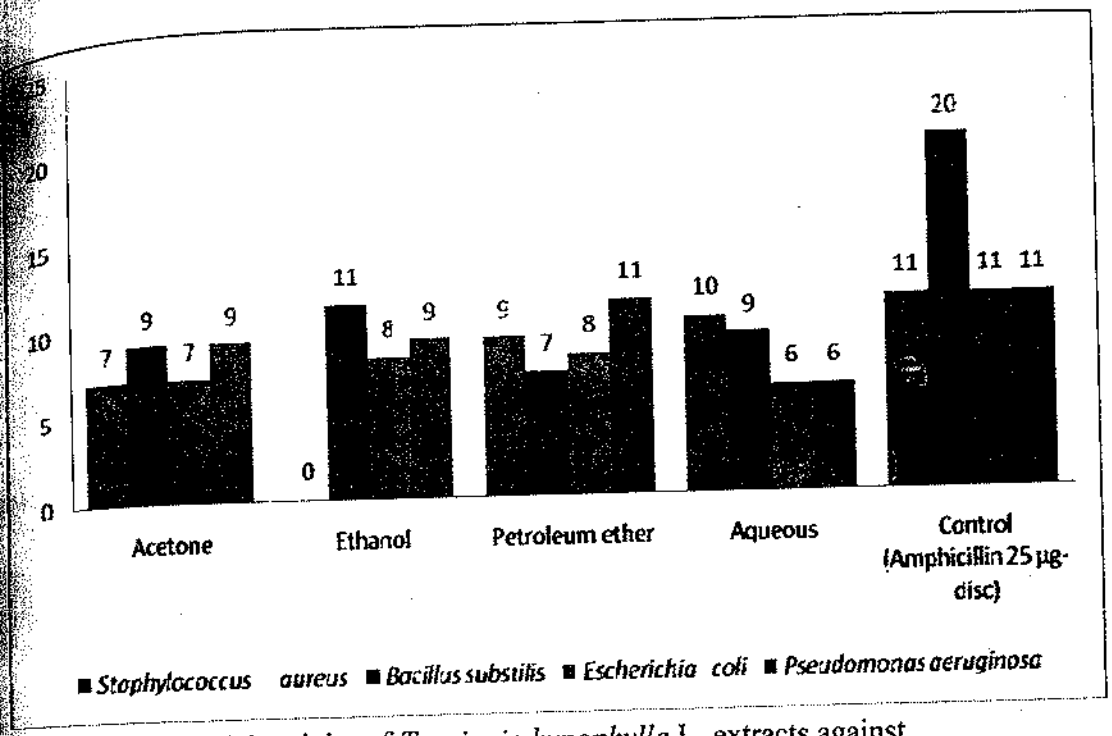
Y- axis : Values of inhibition zone (mm).

ble No. 16 Antibacterial screening of *Targionia hypophylla* L. extracts against test organisms.

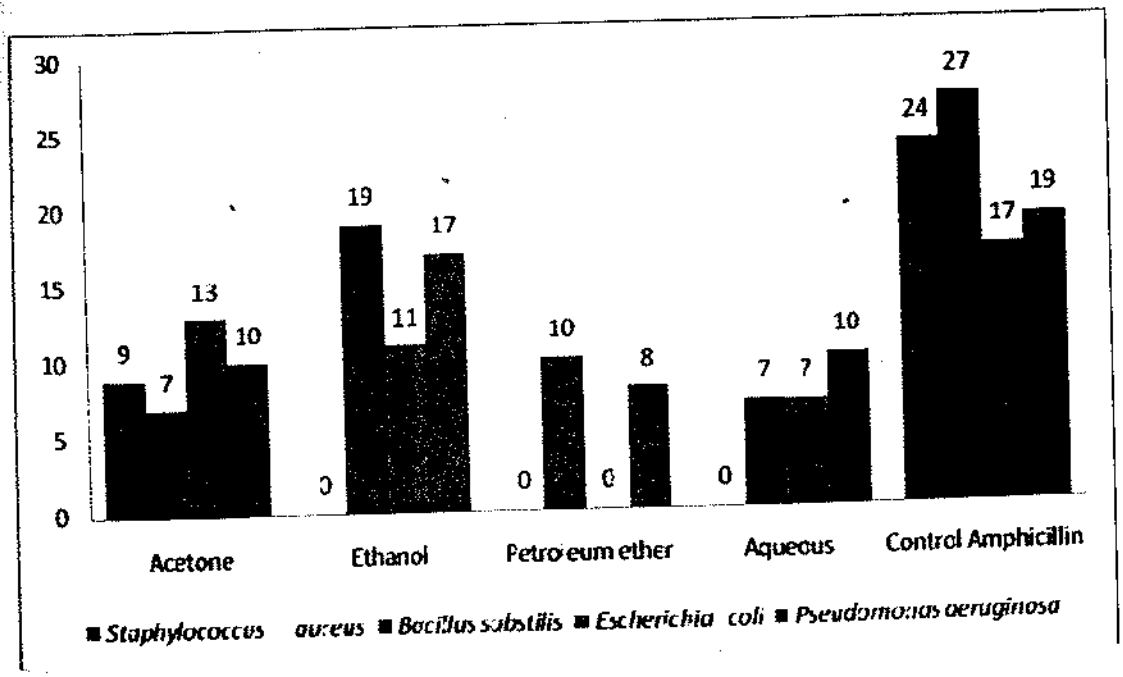
	T. hypophylla L. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Sinhagad	Acetone	7.0	9.0	7.0	9.0
	Ethanol	NI	11.0	8.0	9.0
	Petroleum ether	9.0	7.0	8.0	11.0
	Aqueous	10.0	9.0	6.0	6.0
	Control, (Amphicillin 25 μg^{-disc})	11.0	20.0	11.0	11.0
Rayreshwar	Acetone	9.0	7.0	13.0	10.0
	Ethanol	NI	19.0	11.0	17.0
	Petroleum ether	NI	10.0	NI	8.0
	Aqueous	NI	7.0	7.0	10.0
	Control, (Amphicillin 25 μg^{-disc})	24.0	27.0	17.0	19.0

NI : No Inhibition

31. Antibacterial activity of *Targionia hypophylla* L. extracts against test organisms at Sinhagad.



32. Antibacterial activity of *Targionia hypophylla* L. extracts against test organisms at Rayreshwar.



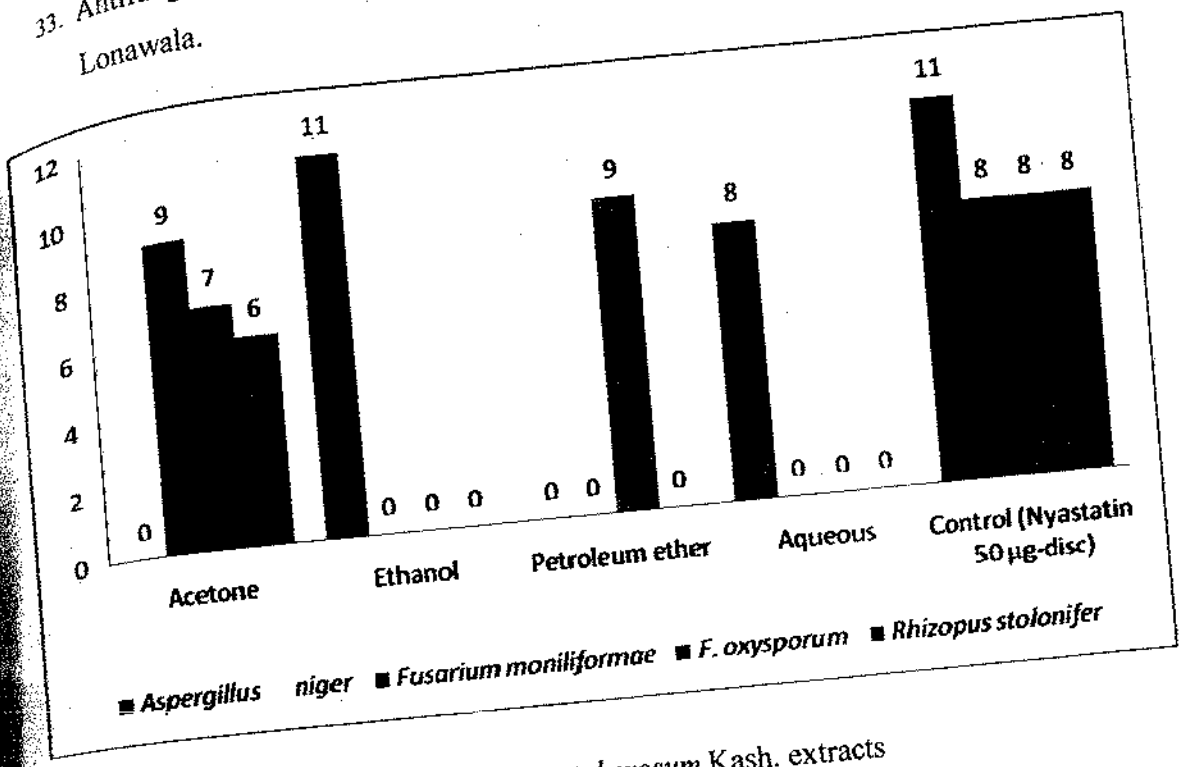
Y- axis : Values of inhibition zone (mm).

Table No.17 Antifungal screening of *Cyathodium tuberosum* Kash. extracts against test organisms.

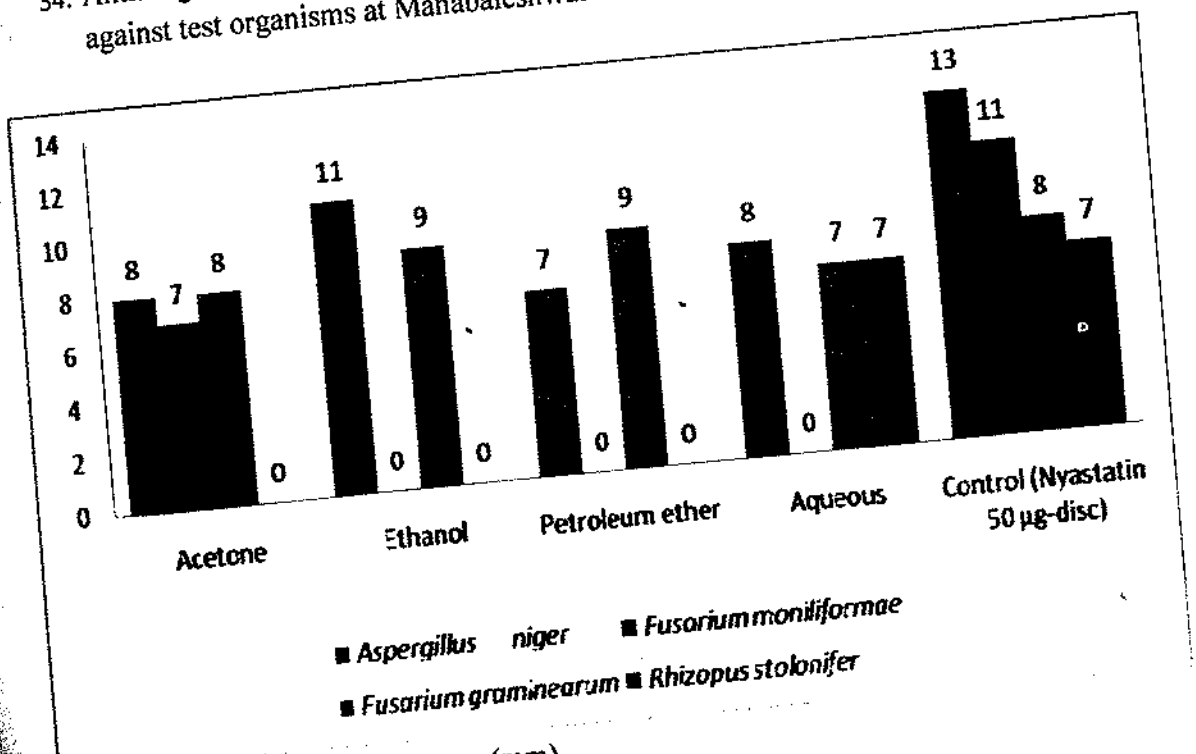
	C. tuberosum Kash. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Lonawala	Acetone	NI	9.0	7.0	6.0
	Ethanol	11.0	NI	NI	NI
	Petroleum ether	NI	NI	9.0	NI
	Aqueous	8.0	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	11.0	8.0	8.0	8.0
Mahabaleshwar	Acetone	8.0	7.0	8.0	NI
	Ethanol	11.0	NI	9.0	NI
	Petroleum ether	7.0	NI	9.0	NI
	Aqueous	8.0	NI	7.0	7.0
	Control, (Nyastatin 50 μg^{-disc})	13.0	11.0	8.0	7.0

NI : No Inhibition

33. Antifungal activity of *Cyathodium tuberosum* Kash. extracts against test organisms at Lonawala.



34. Antifungal activity of *Cyathodium tuberosum* Kash. extracts against test organisms at Mahabaleshwar.



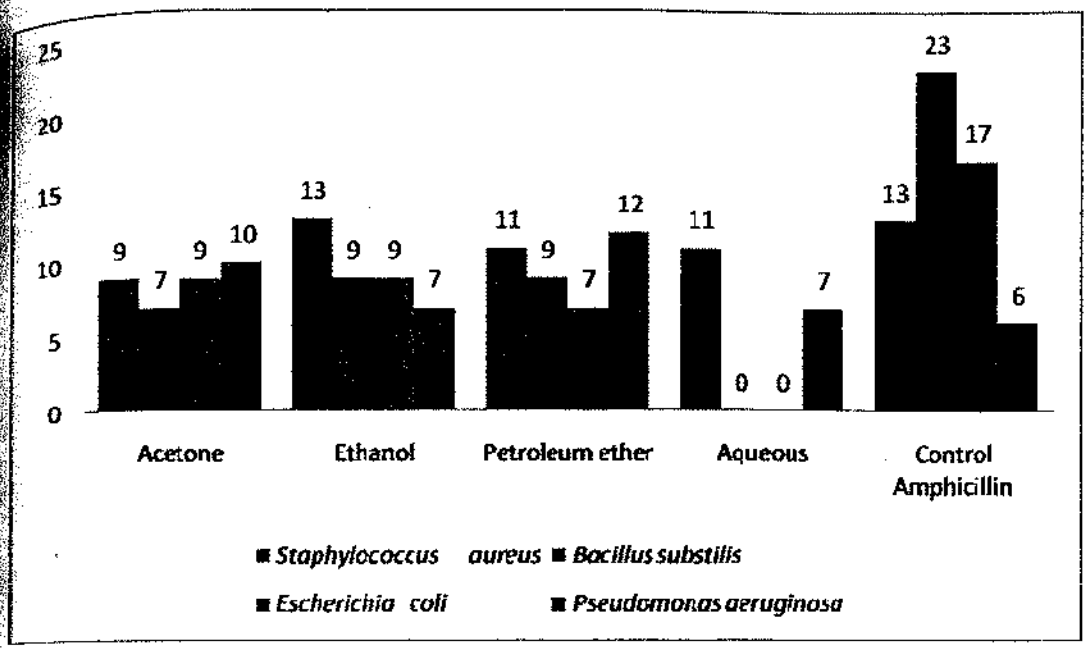
Y- axis : Values of inhibition zone (mm).

Table No. 18 Antibacterial screening of *Cyathodium tuberosum* Kash. extracts against test organisms.

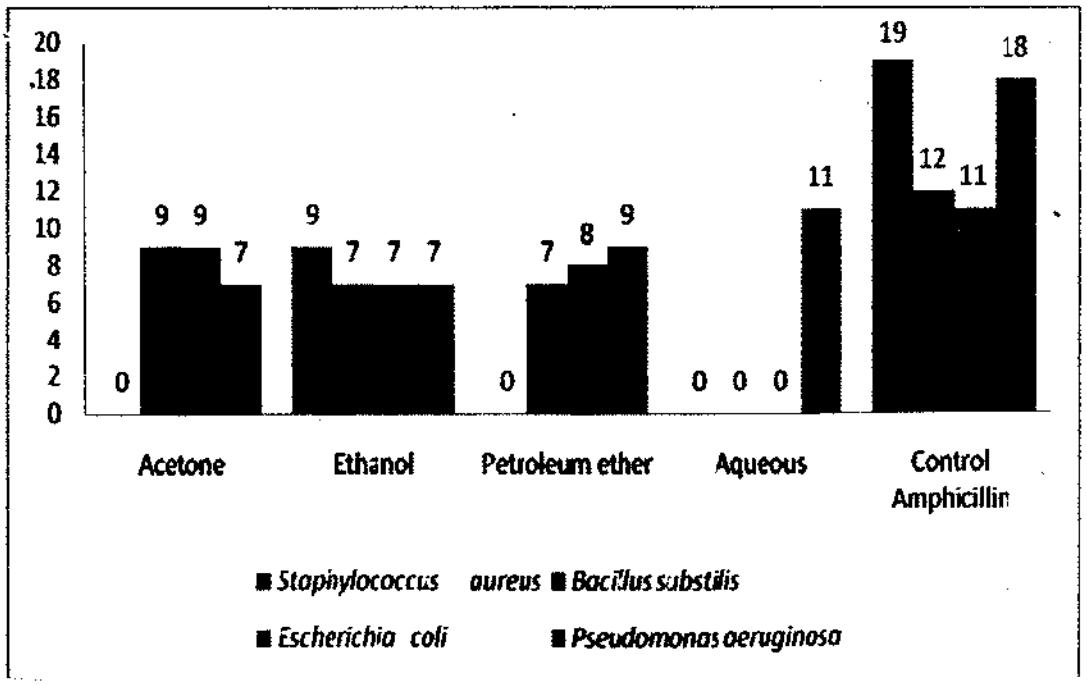
	C. tuberosum Kash. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Lonawala	Acetone	9.0	7.0	9.0	10.0
	Ethanol	13.0	9.0	9.0	7.0
	Petroleum ether	11.0	9.0	7.0	12.0
	Aqueous	11.0	NI	NI	7.0
	Control, (Amphicillin 25 μg^{-disc})	13.0	23.0	17.0	6.0
Mahabaleshwar	Acetone	NI	9.0	9.0	7.0
	Ethanol	9.0	7.0	7.0	7.0
	Petroleum ether	NI	7.0	8.0	9.0
	Aqueous	NI	NI	NI	11.0
	Control, (Amphicillin 25 μg^{-disc})	19.0	12.0	11.0	18.0

NI : No Inhibition

35. Antibacterial activity of *Cyathodium tuberosum* Kash. extracts against test organisms at Lonawala.



36. Antibacterial activity of *Cyathodium tuberosum* Kash. extracts against test organisms at Mahabaleshwar.



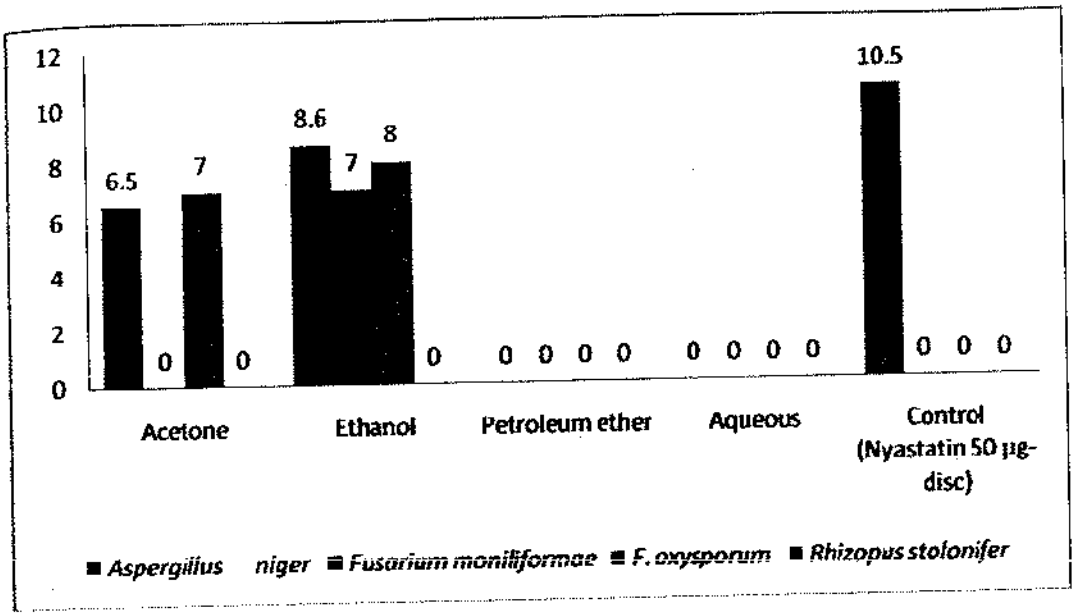
Y- axis : Values of inhibition zone (mm).

ble No. 19 Antifungal screening of *Riccia discolor* L. extracts against test organisms.

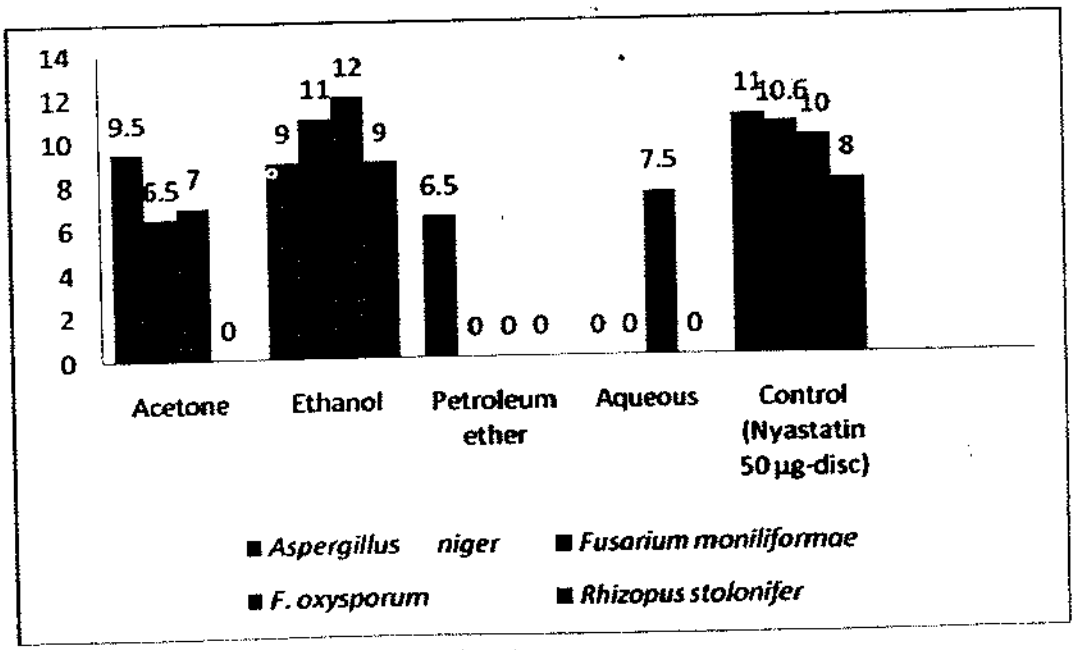
	R. discolor L. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Rajgad	Acetone	6.5	NI	7.0	NI
	Ethanol	8.6	7.0	8.0	NI
	Petroleum ether	NI	NI	NI	NI
	Aqueous	NI	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	10.5	NI	NI	NI
Kas; Satara	Acetone	9.5	6.5	7.0	NI
	Ethanol	9.0	11.0	12.0	9.0
	Petroleum ether	6.5	NI	NI	NI
	Aqueous	NI	NI	7.5	NI
	Control, (Nyastatin 50 μg^{-disc})	11.0	10.6	10.0	8.0

NI : No Inhibition

37. Antifungal screening of *Riccia discolor* L. extracts against test organisms at Rajgad.



38. Antifungal screening of *Riccia discolor* L. extracts against test organisms at Kas; Satara.



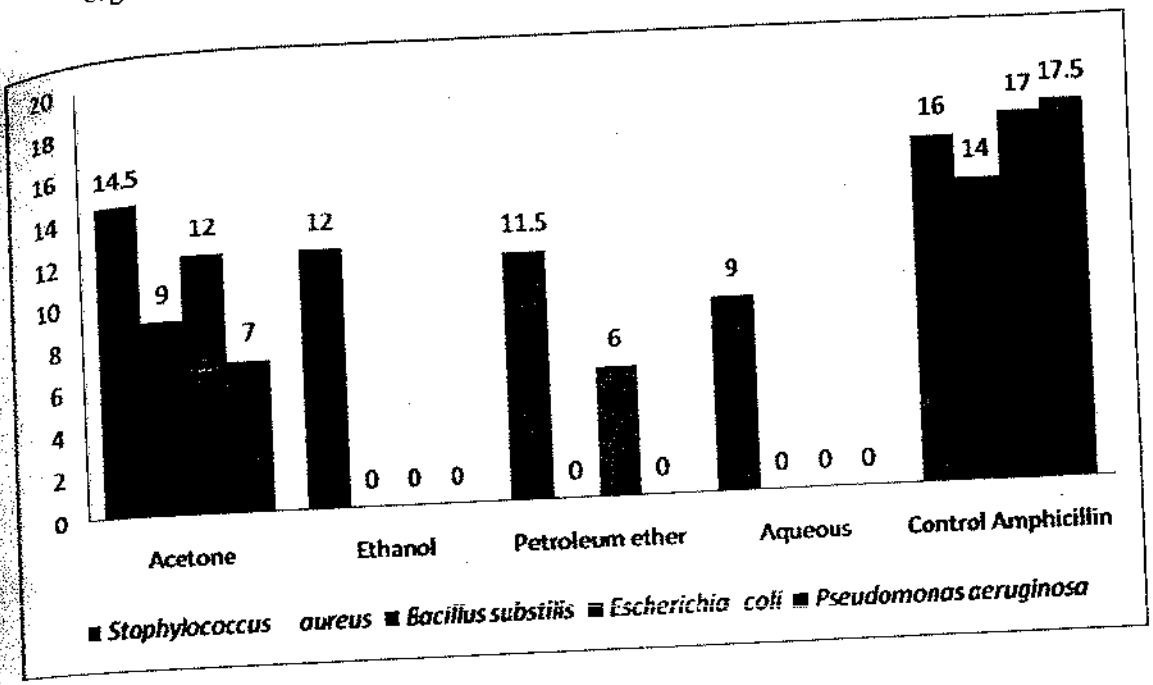
Y- axis : Values of inhibition zone (mm).

Table No. 20 Antibacterial screening of *Riccia discolor* L. extracts against test organisms.

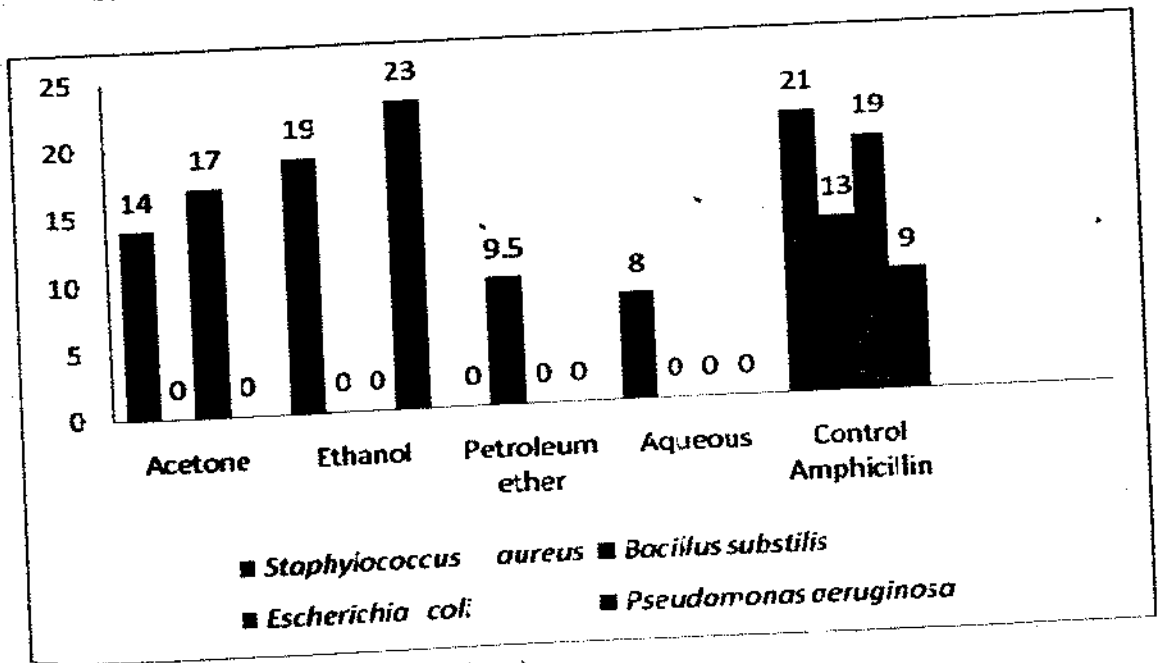
	Nature of *R. discolor* L. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Rajgad	Acetone	14.5	9.0	12.0	7.0
	Ethanol	12.0	NI	NI	NI
	Petroleum ether	11.5	NI	6.0	NI
	Aqueous	9.0	NI	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	16.0	14.0	17.0	17.5
Kas;Satara	Acetone	14.0	NI	17.0	NI
	Ethanol	19.0	NI	NI	23.0
	Petroleum ether	NI	9.5	NI	NI
	Aqueous	8.0	NI	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	21.0	13.0	19.0	9.0

NI : No Inhibition

39. Antibacterial activity of *Riccia discolor* L. extracts against test organisms at Rajgad.



40. Antibacterial activity of *Riccia discolor* L. extracts against test organisms at Kas; Satara.



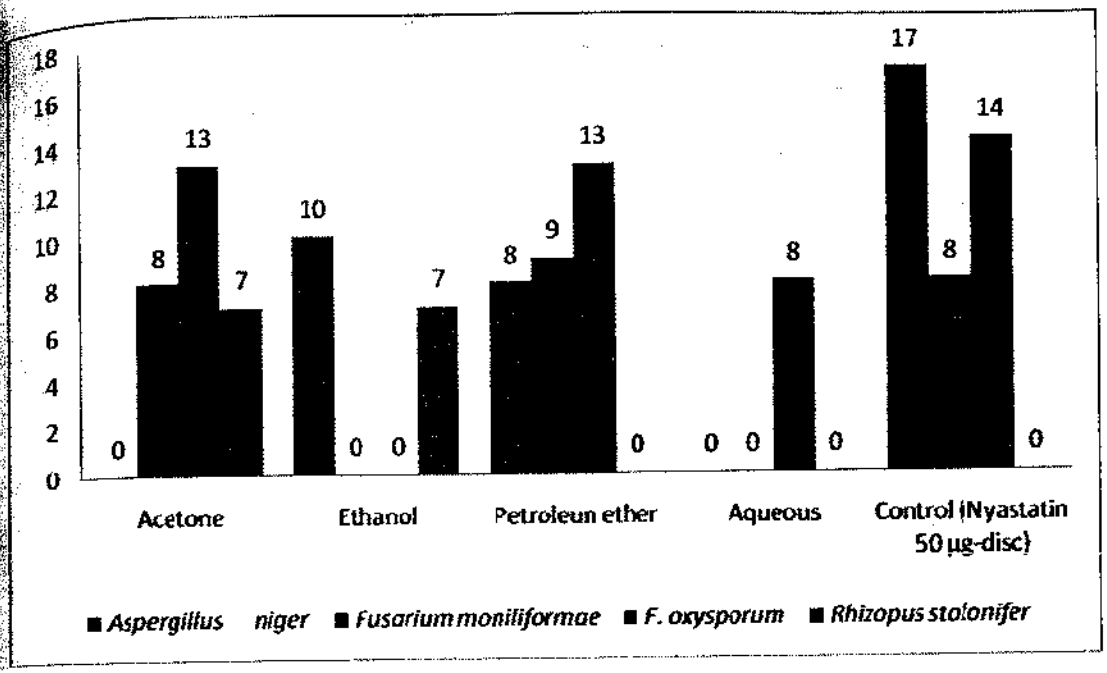
Y- axis : Values of inhibition zone (mm).

Table No. 21 Antifungal screening of *Riccia fluitans* L. extracts against test organisms.

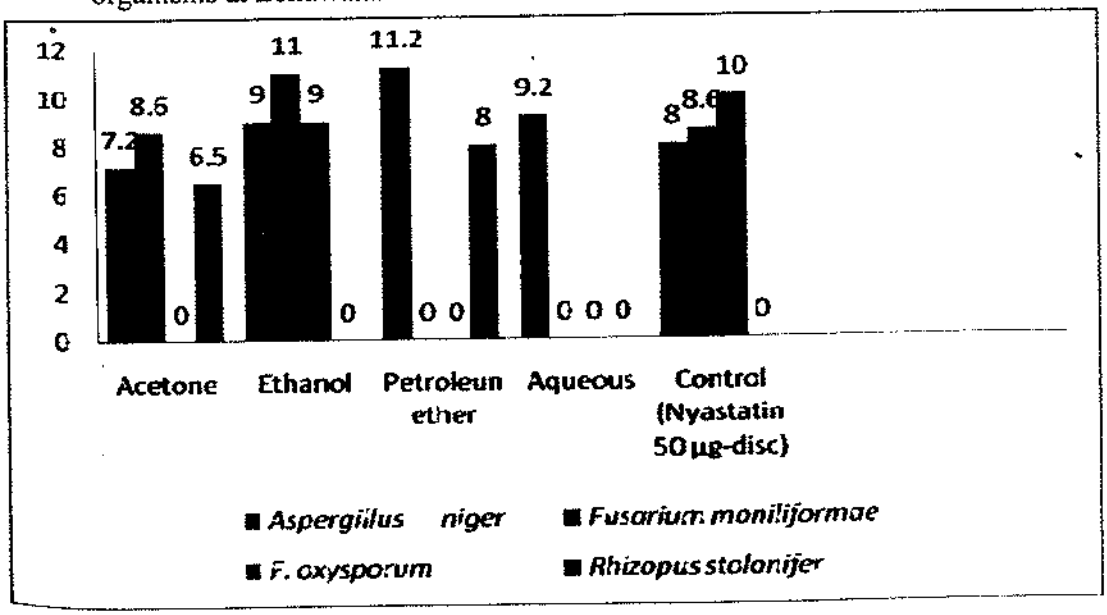
	R. fluitans L. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Kas;Satara	Acetone	NI	8.0	13.0	7.0
	Ethanol	10.0	NI	NI	7.0
	Petroleum ether	8.0	9.0	13.0	NI
	Aqueous	NI	NI	8.0	NI
	Control, (Nyastatin 50 μg^{-disc})	17.0	8.0	14.0	NI
Lonawala	Acetone	7.2	8.6	NI	6.5
	Ethanol	9.0	11.0	9.0	NI
	Petroleum ether	11.2	NI	NI	8.0
	Aqueous	9.2	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	8.0	8.6	10.0	NI

NI : No Inhibition

41. Antifungal activity of *Riccia fluitans* L. extracts against test organisms at Kas; Satara.



42. Antifungal activity of *Riccia fluitans* L. extracts against test organisms at Lonawala.



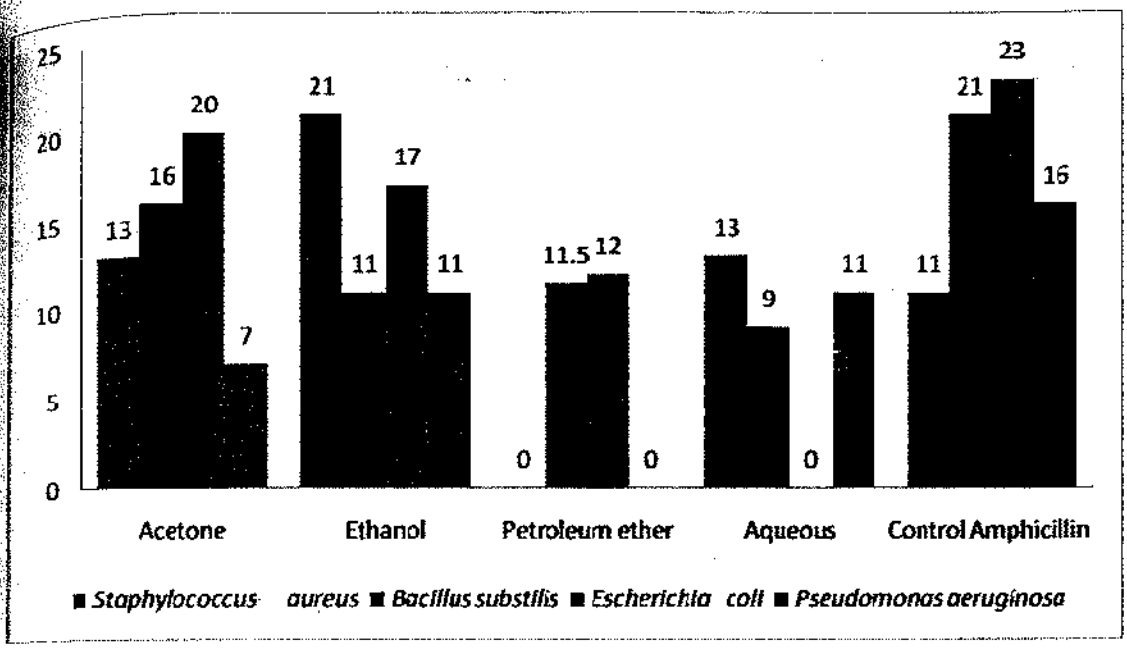
Y- axis : Values of inhibition zone (mm).

Table No. 22 Antibacterial screening of *Riccia fluitans* L. extracts against test organisms.

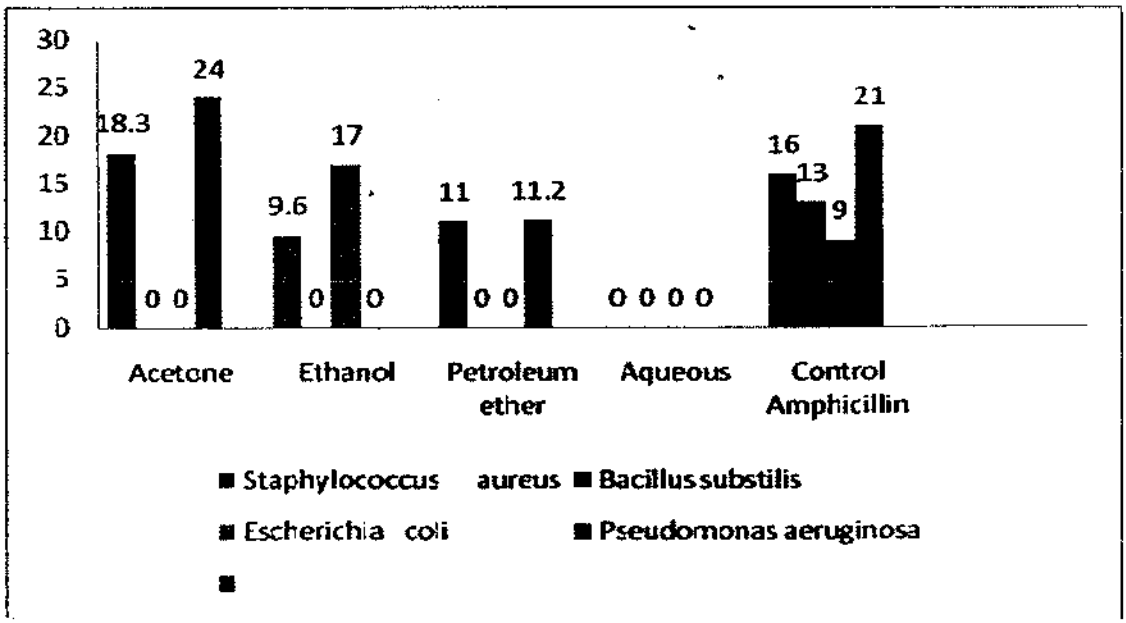
	R. fluitans L. extracts.	Inhibition zone in diameter (mm)			
		S. aureus	*B. substilis*	*E. coli*	*P. aeruginosa*
Kas;Satara	Acetone	13.0	16.0	20.0	7.0
	Ethanol	21.0	11.0	17.0	11.0
	Petroleum ether	NI	11.5	12.0	NI
	Aqueous	13.0	9.0	NI	11.0
	Control, (Amphicillin 25 μg^{-disc})	11.0	21.0	23.0	16.0
Lonawala	Acetone	18.3	NI	NI	24.0
	Ethanol	9.6	NI	17.0	NI
	Petroleum ether	11.0	NI	NI	11.2
	Aqueous	NI	NI	NI	NI
	Control, (Amphicillin 25 μg^{-disc})	16.0	13.0	9.0	21.0

NI : No Inhibition

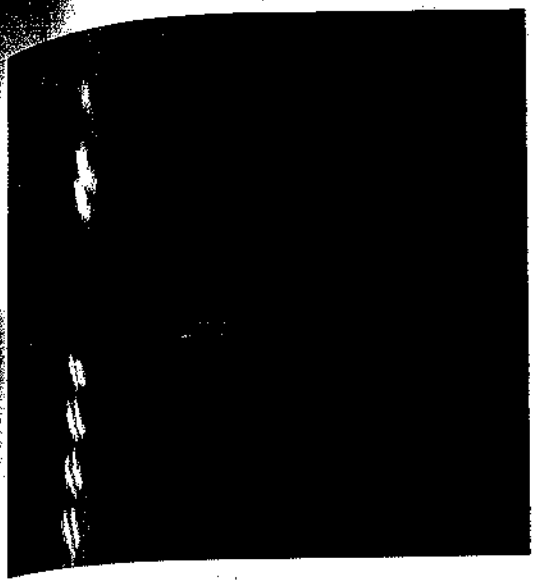
43. Antibacterial activity of *Riccia fluitans* L. extracts against test organisms at Kas; Satara.



44. Antibacterial activity of *Riccia fluitans* L. extracts against test organisms at Lonawala.



Y- axis : Values of inhibition zone (mm).



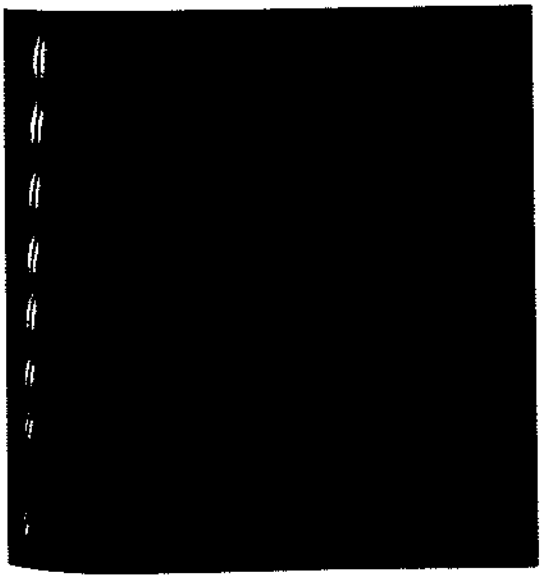
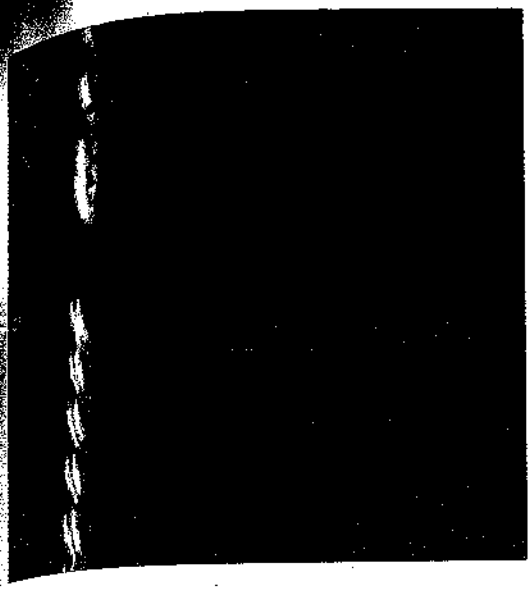
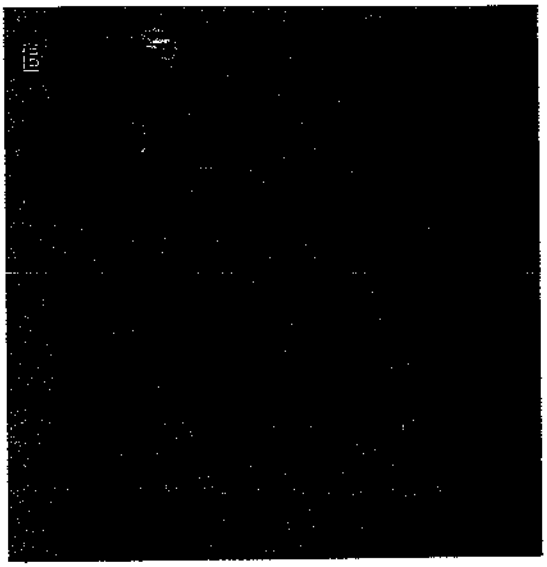
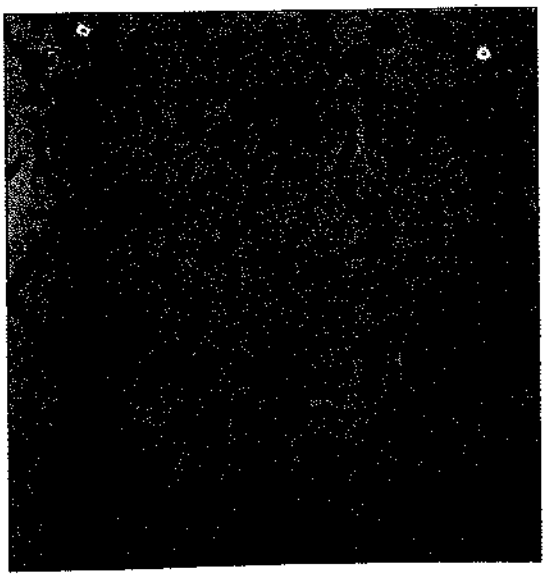


Photo Plates of fungal strains: A. *Aspergillus niger* Van Tieghem.; B. *Fusarium moniliformae* Sheldon.; C. *Fusarium oxysporum* Schlechtendahl.; D. *Rhizopus stolonifer* (Ehrenberg ex fries) Lind.

PLATE - XI







Photoplates of bacterial strains :A. *Bacillus substilis* Ehrenberg.; B. *Escherichia coli* (Migula) Castellani and Chalmers.; C. *Pseudomonas aeruginosa* (Schrroeter)Migula.; D. *Staphylococcus aureus* Rosenbach.

PLATE - XII

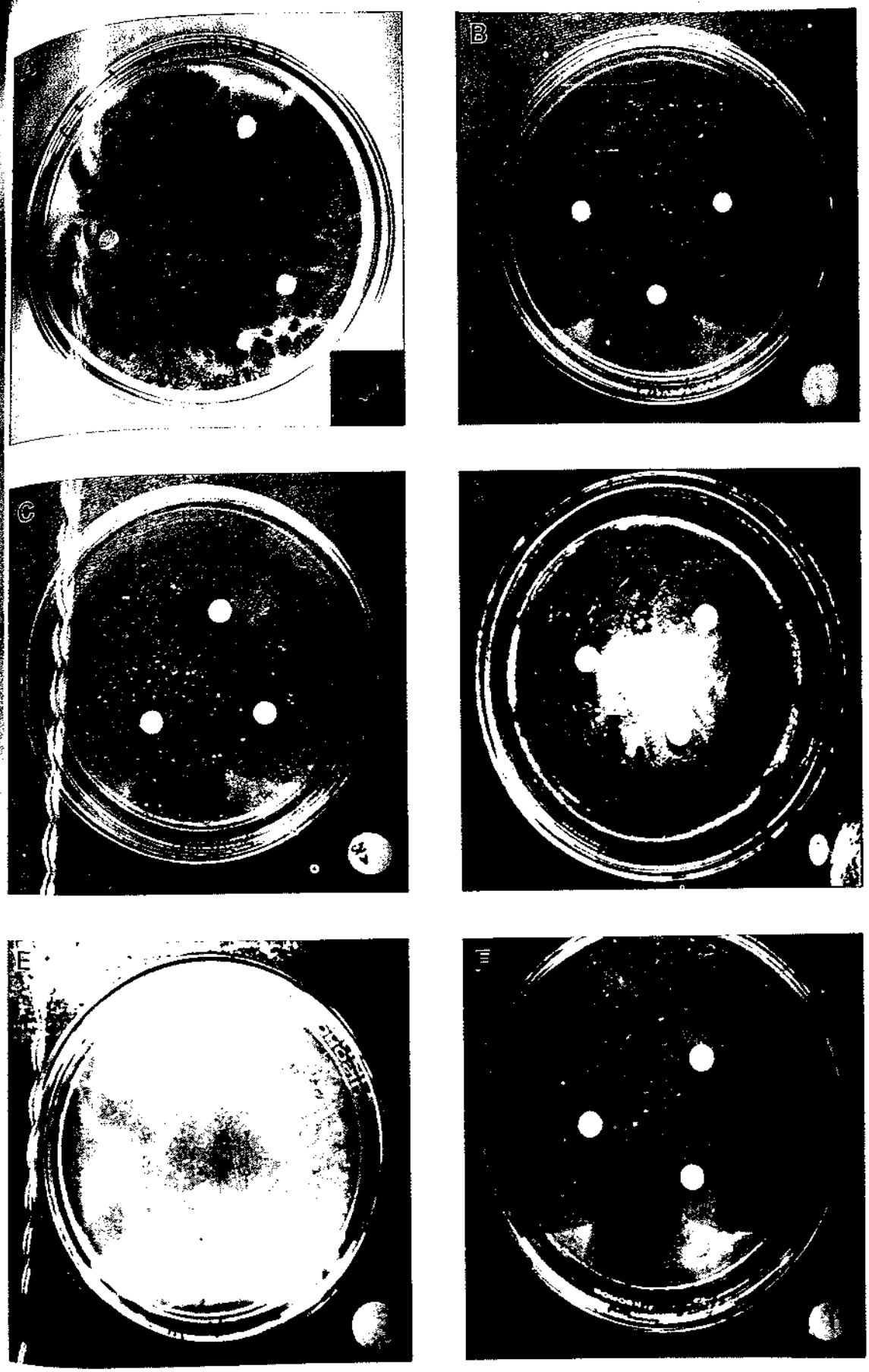


Photo Plates of inhibitory activity: A. ***Fossombronia indica*** St. ethanol extract against *Aspergillus* ***niger;*** B. *Sewardiella tuberifera* Kash. petroleum ether extract against ***E. coli;*** C. *Exormotheca tuberifera* Kash. extract against ***E. coli;*** D. Antibiotic amphicillin against *S. aureus*.; E. ***Asterella*** *angusta* St. aqueous extract against *S.* ***aureus;*** F. *Plagiochasma articulatum* Kash. extract against *S.* ***aureus.***

PLATE - XIII

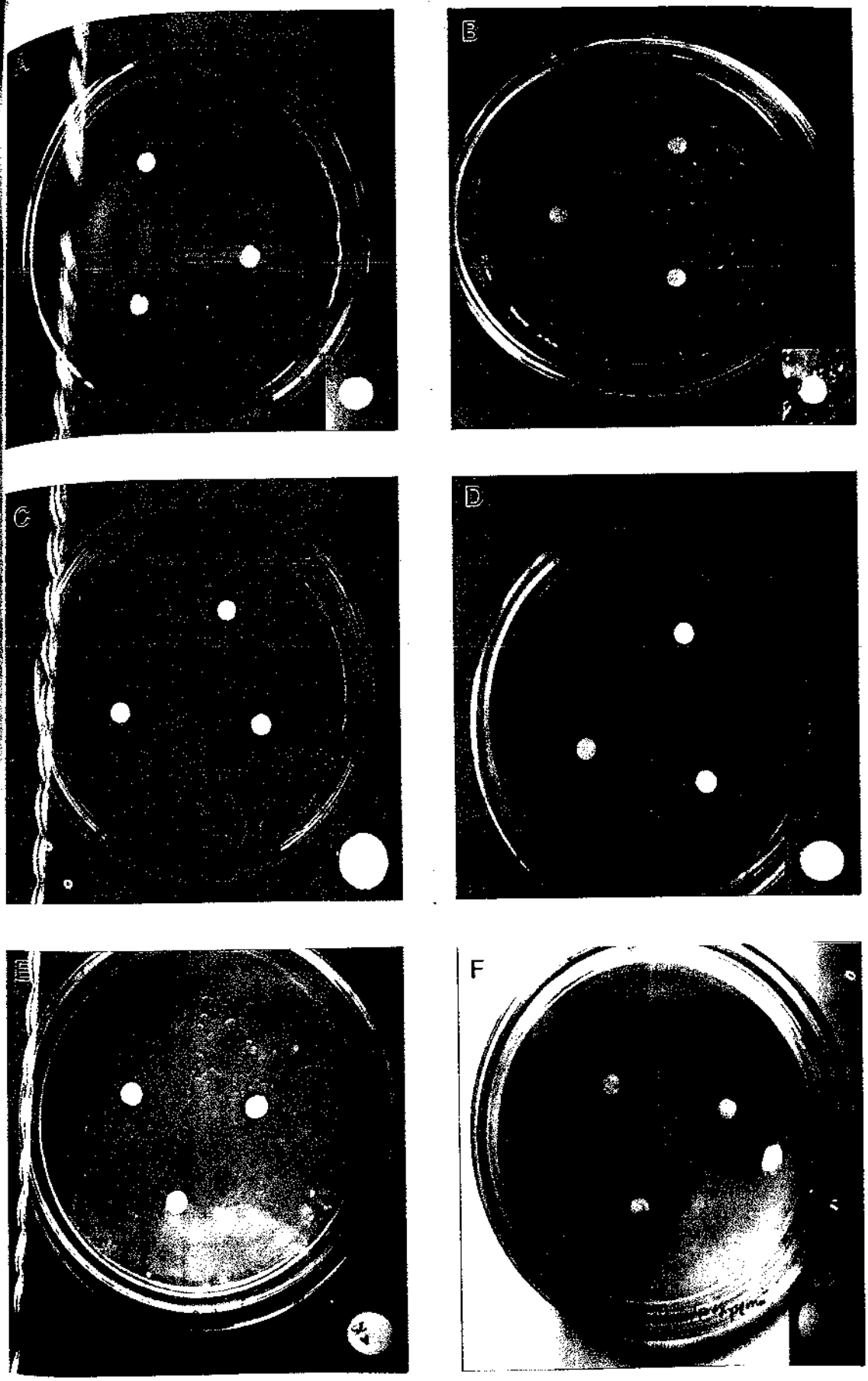
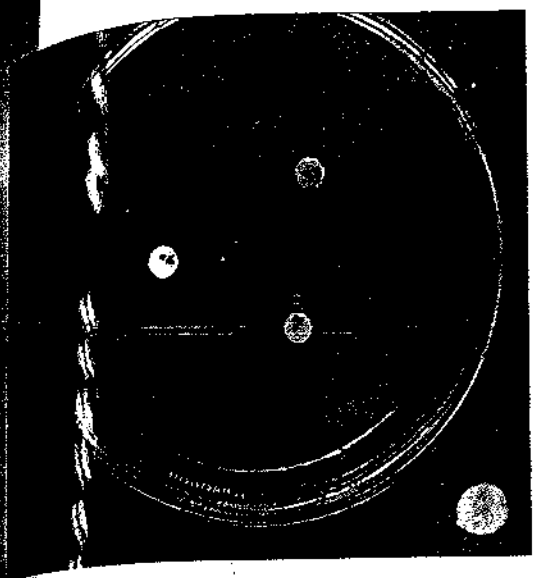
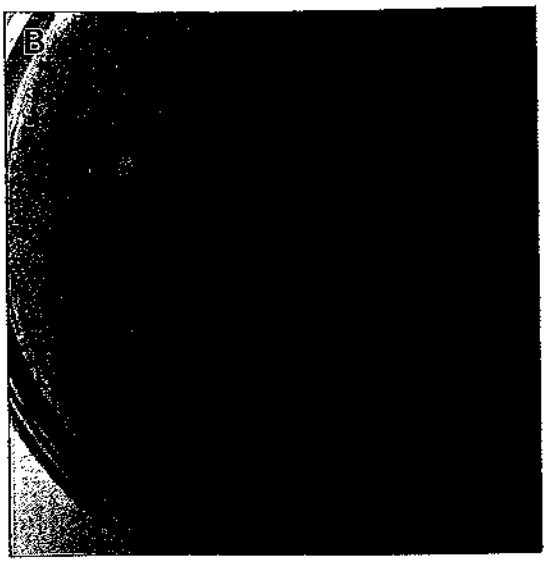


Photo Plates of inhibitory activity : A. *Plagiochasma appendiculatum* Kash. methanol extract against *Fusarium moniliforme*; B. *P. appendiculatum* Kash. methanol extract against *F. graminearum*; C. *P. simulensis* Kash. n-butanol extract against *Aspergillus niger*; D. *P. simulensis* Kash. methanol extract against *A. niger*; E. *Targionia hypophylla* (Mich.) L. petroleum ether extract against *F. oxysporum*; F. *Cyathodium tuberosum* Kash. petroleum ether extract against *F. oxysporum*.

PLATE - XIV





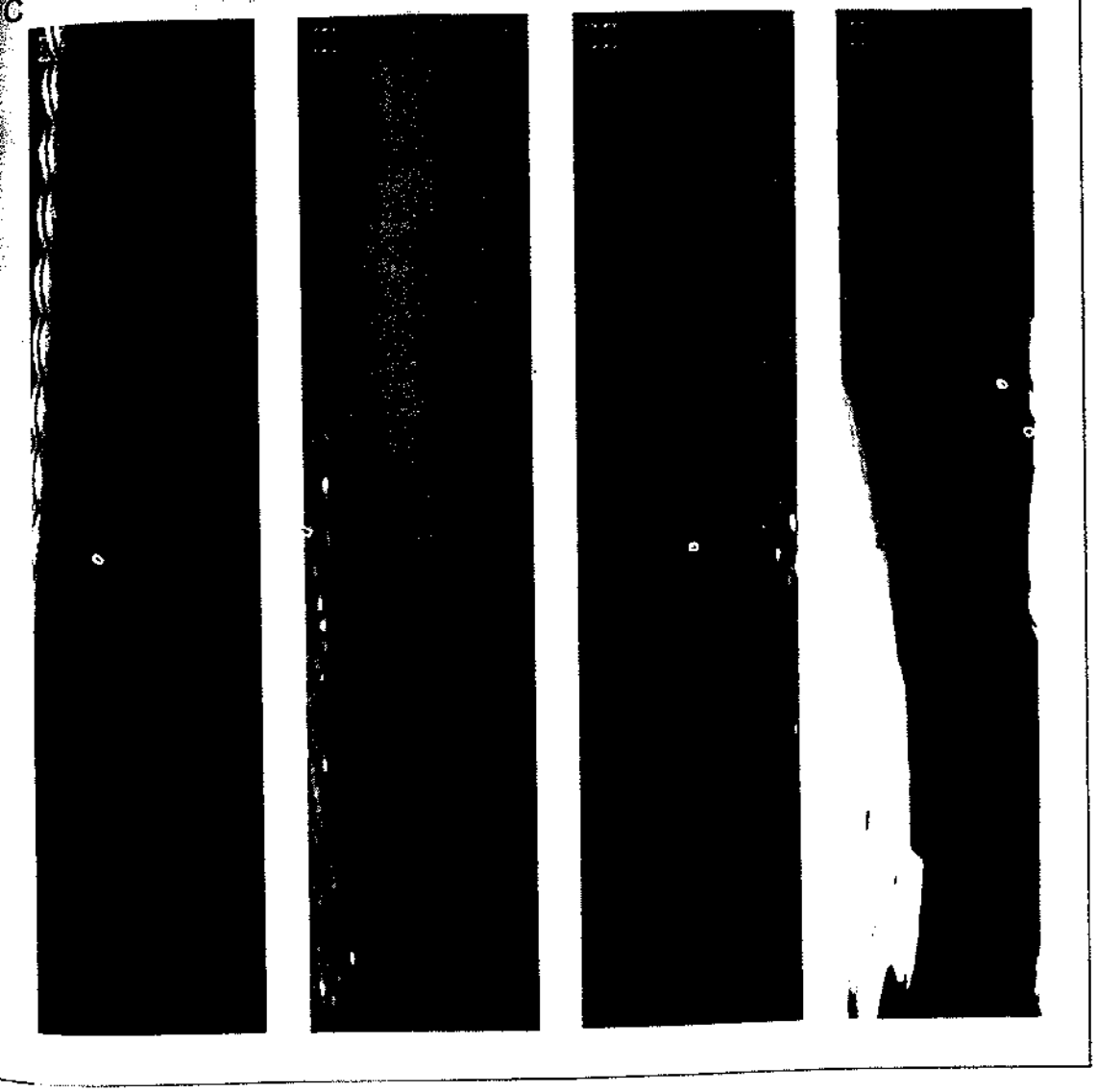


Photo Plates of inhibitory activity: A. *Riccia discolar* L. aqueous extract against *S. aureus*;
B. *R. fluitans* L. ethanol extract against S. aureus.
Photo Plates of TLC : C, I. *Asterella angusta* St. extracts; C, II. *Plagiochasma simulensis* Kash. extracts;
C, III. *Targionia hypophylla* (Mich.) L. extracts; C, IV. *Cyathodium tuberosum* Kash. extracts.

We have evaluated the antimicrobial properties of thalloid liverworts extracts at ...tern Ghats of Maharashtra. The antimicrobial activity is due to presence of secondary ...bolites or bioactive compounds. Many plants, animals, fungi and microbes possess a ...variety of chemical defensive and offensive mechanisms as part of their survival ...egy thus, it is not to surprising to learn that nearly half of all medical prescriptions ...ain ingredients derived from natural sources of which 20-25% are of plant origin (Farderosian and Liberti, 1988). A natural product is an entire organic, or any part of it, ...an isolated chemical substances. In Vitro antimicrobial activity of *Brachythecium ...pestre* and *Eurhynchium pulchellum* extracts examined (Yayintas and Yapici, 2009). ...bioactive substances of *Atricum* are considered to be polyphenolic compounds ...silc *et al.*, 1999). *Atricum* labels are reported to be seen on Chinese medicines ...marily as antibacterial and anti-inflammatory agents (Glime, 2007). *Atricum selwynii* ...*Sphagnum pulustre* have also shown activity against a variety of Gram-positive and ...ram-negative bacterial strains (Vanwagen and Cardellina, 1986). *Hylocomium ...lendens* has shown antibiotic activity against nine Gram-positive bacteria (Kang *et al.*, ...007). However, it is well known by bis (bibenzyls)- various type of marchantin, some of ...which besides antimicrobial, are known to have anti-cancer affect (Asakawa, 2008; Chong *et al.*, 2006). The Marchantiaceae members extensively used to treat tumefaction, to protect the liver and to treat hepatitis, being also used as antipyretics (Chobot *et al.*, 2006; Harris, 2008). Fourteen crude methanolic and ethanolic extracts of bryophytes from South Western British Columbia were screened for antibiotic activity against three bacterial strains (Russell, 2010). Different organic solvents and aqueous extracts of thalloid exhibits variable inhibitory activity against target organisms in this study. According to previous literature, liverworts species should have antibiotic activity against *B. substilis* (Zhu *et al.*, 2006). Novel polycyclic benzo-napthoxanthinone; ohioensins A, B, C, D and E through fractionation guided by 9 KB and 9 PS bioassay activity from samples of *Polytrichum ohioense* Ren. & Card. (Zheng *et al.*, 1989). The

characterization by RP-HPLC (High Pressure Liquid Chromatography) determines the presence of phenolic compounds: vanillic acid, syringic acid, chlorogenic acid, gallic acid, 3-4 hydroxybenzoic acid, caffieic acid, P-coumaric acid, and salicylic acid and these components could be responsible for the antibacterial activity (Montenegro *et al.*, 2009). The detectable antibacterial compounds are present in most taxa of liverworts (McCutcheon *et al.*, 1995). Castaldo Cobiachi, (1988) showed high antibacterial activity of *Conocephalum conicum* against pathogenic bacteria. McCleary and Walkington, (1966) considered that non-ionized organic acids and polyphenolic compounds might contribute to the antibiotic properties of bryophytes. With connection of these studies our report indicates that the antimicrobial activities of thalloid liverworts.

The first in vivo experiments have been performed at Boon University, alcoholic extracts of all twenty bryophytes used and had an effect on a variety of crop infected different fungi. Based on these results, commercial products from bryophytes have been developed and are sold in Germany (Frahm, 2004). However, a patent has been obtained to cure fungal infections of horses with bryophyte extract. The bryophytes are considered to be an excellent system for experimental studies in plant physiology and phytochemistry. The GC/MS analysis of ether extract of several Marchantiophyta species indicated that each liverwort species, produce own characteristic antibiotic compounds (Ludwiczuk and Asakawa, 2010). The chlorinated cyclic bisbibenzyls of isoplagiochin type are the first verified halometabolite from bryophytes (Speicher *et al.*, 2003). Liverwort *Lunularia cruciata*, a mediterranean atlantic species, expresses antimicrobial and to less extent, antifungal activities (Basile *et al.*, 1998). The Borneo *Frullania serrata* Gott. L. and Nees like many other species belonging to chemotype I (sesquiterpene lactone type) produce eudesmane type sesquiterpene lactone. All of these compounds possess an alpha-methylene and beta-butyrolactone group are allergy-inducing substances (Asakawa, 1995, 2001, 2008). Methyl benzoates with prenyl ether group, diterpenoids and flavonoids were detected in these liverwort species (Asakawa, 1981;

Perry *et al.* 1996; Beak *et al.* 1998; Nagashima *et al.*, 2003). Sometimes, species of liverworts from different locations produce compound characteristics only for one environment. Taiwanese *Trichocolea pluma* produce labdane diterpene alcohol, not occurs in Tahitian and Borneo specimens (Chang and Wu, 1987). The antimicrobial activity of bryophytes depends on the physiological age of the species, season of the collection and ecological conditions in which they are growing (Joshi *et al.*, 1990). Our recent report on antimicrobial screening gives variable activities at different localities.

A. angusta St. extracts from Lonawala, has strong antimicrobial activities. The biologically active substance has demonstrated significant experimental results to inhabiting tested organisms. The antibiotic activity may indicates that the variation in season, in soil type. The *P. appendiculatum* L. possesses significant antibacterial and antifungal activities (Singh *et al.* 2006). Here, the different organic solvent extracts of *Fossombronia indica* St. at Purandar exhibits the greater antimicrobial properties. Several liverworts like *Bazzania, Frullania, Marchantia, Plagiochilla, Porella* and *Radula* and their extracts have been used for testing antimicrobial activities. In vitro antimicrobial activity of *Brachythecium compestre* and *Eurhynchium pulchellum* extract studied (Yayintas and Yapici, 2009). Antifungal compounds were isolated from New Zealand liverwort *Plagiochila faciculata* (Lorimer and Perry, 1993). Cinnamolide from *Porella* and *Makinoa* showed antibiotic activity against a few species of fungal dermatophytes (Subramanian and Subhisha, 2005). Thalloid liverworts viz. *F. indica* St., *S. tuberifera* Kash., *E. tuberifera* Kash., *A. angusta* St., *P. articulatum* Kash., *P. appendiculatum* L., *P. simulensis* Kash., *T. hypophylla* L., *C. tuberosum* Kash., *Riccia discolar* L., and *R. fluitans* L., and their extracts exhibits stronger antifungal and antibacterial activities. Acetone and ethanol extracts of *P. appendiculatum* L.et L. possess strongest antimicrobial activity while, the petroleum ether and aqueous extract showed lower activity. The screening of thalloids for antibacterial and antifungal activities are due to potentially rich source of antimicrobial agents. It is hoped that, this piece of work will

piece of work will unravel many intricate problems pertaining to antibiotic properties of liverworts and will also give a guideline to future work.

In-*vitro* antifungal activity of *Pallavicina lyelli* was studied (Subhisha and Subramanian, 2005). Different organic solvent extracts of *P. lyelli* showed varying levels of activity against the test fungi. Our results indicates that varying levels of activity against tested organisms. Liverworts and mosses screened to determine their antimicrobial activity against selected bacterial and fungal species and recorded promising effect on growth inhibition of test organisms (Bodade *et al.*, 2008).

The degree of antibiotic activity depends on the age of plant therefore, vegetative and reproductive stage material will collected for screening preparation. Bryophytes are considered to be an excellent system for experimental studies in plant physiology and biochemistry (Chopra and Kumra, 1988). The use of herbal remedies are very common in Asia for diabetes and its complications (Zheng, 2004). Inhibitory activity towards alpha-glycosidase by compounds 1, 3-, 6 and trifarienol A (8), from the Malaysian *Cheilolejeunia trifaria* was evaluated (Hashimoto *et al.*, 1995). McCleary and Walkington, (1966) considered that, non- ionized organic acids and polyphenolic compounds might contribute to the antibiotic properties of bryophytes. Madsen and Pates, (1952) found that the inhibition of microorganisms by product of bryophytes. Belcik and Wiegner, (1980) reported the antimicrobial activity in extracts of the liverworts *Pallavicinia* and *Reboulia*, and from *Porella* (Isoe, 1983). A dichloromethane and methanol extract the liverworts *Bazzania trilobata* showed antifungal activity against the phytopathogenic fungi (Jochen *et al.*, 2004). Biologically active terpenoids may cause allergic effects against peoples (Ando and Matsuo, 1984). Asakawa, (1981) has shown that several compounds from leafy liverworts exhibit anti leukemic activity. A large number of liverworts are used as medicinal plants to treat burns, bruises and external wounds (Asakawa, 1994). According Adamek and coworkers, (1976) some peat

preparations hold some promise against some type of human cancer. Diplophyllin isolated from *Diplophyllum albicans*, had significant activity against human epidermoid carcinoma (Ohta *et al.*, 1977).

Thalloid liverworts from Western Ghats of Maharashtra have reported to deter the growth of organisms such as fungi and bacteria. High occurrence of antibacterial activity for extracts of *Barbula* species studied (Gupta and Singh, 1971). The isolated three prenyl bibenzyls could inhibit growth of *Staphylococcus aureus* (Asakawa, 1982). Flavonoids shown to have pronounced antibacterial effects against *Enterobacter cloceae*, *E. aerogenes*, and *Pseudomonas aeruginosa* (Basile *et al.*, 1999). Liverwort *Plagiochasma japonica* and *Marchantia tonosa* exhibit antitumor and antimicrobial activity (Lahlou *et al.*, 2000). In vitro activity of eight aliphatic long chain aldehydes have a broad antimicrobial spectrum and show similar activity against gram positive and gram negative microorganisms (Giuseppe *et al.*, 2001). Ichikawa, (1982) found the antimicrobial activity of mosses completely inhibited the growth of rice blast fungus *Pyricularia oryzae*. Plagichin-E, a macrocyclic bis (bibenzyl) isolated from liverwort *Marchantia polymorpha*, has been reported to have antifungal activity, and resistance reversal effect on *Candida albicans* (Ling MoiSun *et al.*, 2009). In the antimicrobial screening, four fungal strains used *viz. Aspergillus niger* (NCIM 507), *Fusarium moniliformae* (NCIM 1276), *Fusarium oxysporum* (NCIM 1072), and *Rhizopus stolonifer* (NCIM 1139) and four bacterial strains *viz. Bacillus substilis* (NCIM 2697), *Escherichia coli* (NCIM 2067), *Pseudomonas aeruginosa* (NCIM 2200), *Staphylococcus aureus* (NCIM 2492). A dichloromethane and methanol extract of the *Bazzania trilobata* showed antifungal activity against the phytopathogenic fungi (Jochen *et al.*, 2004). Spjut, (2006) reported the first discovery of significant biological activity of moss *Polytrichum ohioense*. *Coenocephalum conicum* and its opigenin acts as potential amplitudes, and its flavonoids considered to prevent a number of human diseases (Bozena *et al.*, 2007). The

antimicrobial activities of thalloid liverworts extracts has not been previously reported from Western Ghats of Maharashtra.

Our results have evaluated as first time report on antimicrobial properties of thalloid liverworts extracts from Western Ghats of Maharashtra. This study indicates that the thalloid liverworts might possess a novel bioactive compounds which has an inhibitory effect against the fungal and bacterial strains.

Chapter. 5 F. Characterization of selected thalloid liverworts extracts.

A) *Asterella angusta* St.

a) Susceptibility of microorganisms to fresh and stored *Asterella angusta* St. extracts.

Susceptibility of extracts was evaluated and is documented bellow -

Susceptibility of test organisms to fresh and stored *A. angusta* St. extract.

Nature of extract	Inhibition zone (mm) against target organisms			
	A	B	C	D
Fresh extract	12	NI	9	NI
Stored extract	11	NI	9	NI

NI : No Inhibition

A- *Aspergillus niger*, B- *Fusarium moniliformae*,
C- *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

b) Effect of pH on activity and stability of bioactive compounds from *A. angusta* St. extracts.

Stability of bioactive compounds from extract, at different pH was checked by pre-incubating for 1 hr in phosphate buffer at different pH ranging from 5.7 to 8.0. The bioactive extract was quite stable within this pH range as tested against target organisms such as *A. niger*, *F. moniliformae*, *F. oxysporum*, and *R. stolonifer*. After incubation of bioactive extracts at pH in range of 5.7 to 8, the maximum residual activity was observed at pH range 6.8-8.0, where as the activity decreased at pH 6.8 and bellow.

Effect of pH on activity of bioactive compounds from *A. angusta* St. extracts.

pH	Inhibition zone (mm) against target organisms			
	A	B	C	D
5.7	11	NI	NI	NI
6.8	12	NI	NI	NI
7.3	12	NI	9.5	NI
8.0	11	NI	8.0	NI

A- *Aspergillus niger*, B- *Fusarium moniliformae*,
C - *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

c) Thermal stability of bioactive components from *A. angusta* St. extracts.

Since the activity of bioactive extracts was quite stable at 4°C, experiments were also conducted to see the effect of elevated temperature on stability of bioactive compounds. For this purpose, the bioactive extracts was kept at various temperature (30- 60°C) for one hour. The bioactive compounds was stable at different temperatures.

Thermal stability of bioactive compounds from *A. angusta* St. extracts.

Temperature (°C)	Inhibition zone diameter (mm) against target organisms	
	A	C
30	12	9.0
40	12	9.0
50	11	8.0
60	11	8.0

A- *Aspergillus niger*, C - *Fusarium oxysporum*
Data values represent average of three replicates.

d) Effect of detergents on bioactivity of *A. angusta* St. extracts against target organisms.

Effect of various detergents *viz.* Tween-20 and cetrimide on activity of bioactive components from extracts was determined by mixing them with extracts, and incubating for 6 hrs at 30°C. The activity was checked against target organisms. Detergents added to distilled water are used as control to check the effect of detergents themselves on *F. oxysporum*. The bioactive compounds alone showed 9 mm inhibition zone diameter against *F. oxysporum*, while inhibition zone diameter obtained with mixer of bioactive extract and detergents. This clearly indicated that, there was no significant loss of antifungal activity of bioactive compounds after treatment with detergents.

Effect of detergents on antibiotic activity of *A. angusta* St. extracts against target organisms.

Detergent	Inhibition zone (mm) against target organisms	
	Without bioactive extracts	With bioactive extracts
	A	C
Tween-20	11	9
Cetrimide	11	10

A- *Aspergillus niger*, C - *Fusarium oxysporium* .
The bioactive extracts concentration 100 µl/ml.
Data values represent average of three replicates.

e) Effect of enzymes on activity of bioactive components from *A. angusta* St. extracts against target organisms.

Sensitivity of bioactive components from extracts to enzymes like Protease K, and Lipase was evaluated. The bioactive compounds of extract was mixed with respective enzymes and incubated for 3 hrs. and activity of bioactive components was assayed against *A. niger* and *F. oxysporium*. The antibiotic components alone showed 12 mm and

9 mm inhibition zone diameter against *A. niger* and *F. oxysporium*, while inhibition zone diameter obtained with mixture of bioactive extracts and enzyme ranged between 11 to 12 mm in case of *A. niger* and 9 to 10 mm in case of *F. oxysporum*. This clearly indicated that there was no significant loss of antifungal activity of bioactive extract after treatment with enzymes.

Effect of enzymes on the activity of the bioactive components from *A. angusta* St. extracts against target organisms.

Test	Inhibition zone (mm) against target organisms	
	A	C
Protease K + bioactive extract	12	10
Lipase + bioactive extract	11	9
Antibiotics	12	10

A- *Aspergillus niger*, C - *Fusarium oxysporum*
Data values represent average of three replicates.

f) Solubility of bioactive components from *A. angusta* St. extracts.

A. angusta St. dry extract powder is soluble in each organic solvents *viz.* acetone, ethanol, petroleum ether, and distilled water.

g) Determination of shelf life of bioactive compounds from *A. angusta* St. extracts.

To determine the shelf life of bioactive components from extracts. The extracts was stored at 4°C in different ampoules for 1 week, 1 month, 2 month, 3 month. After the specified storage period, antifungal activity of the extract was determined by 'disc diffusion assay' method. The bioactive components were stable for a period of 3 months at 4°C.

Effect of shelf life of bioactive components from *A. angusta* St. extracts against target organisms.

Incubation time	Inhibition zone (mm) against target organisms			
	A	B	C	D
1 week	12.5	NI	9.0	NI
1 month	12.0	NI	8.5	NI
2 month	11.0	NI	8.0	NI
3 month	11.0	NI	8.0	NI

A- *Aspergillus niger*, B- *Fusarium moniliformae*,
C- *F. oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

h) Thin Layer Chromatography (TLC) of bioactive compounds from *A. angusta* St. extracts.

Liverwort *A. angusta* St. gives good separation with hexane, resolved single component.

TLC results of acetone extracts of *A. angusta* St. (Vegetative stage).

Species	Solvent system	No. of components	Rf value
A. angusta St.	1	1	0.179

Solvent systems - 1. Hexane 2. Hexane & ethyl acetate.

TLC results of acetone extracts of *A. angusta* St. (Reproductive stage).

Species	Solvent system	No. of components	Rf value
A. angusta St.	1	1	0.191

Solvent systems - 1. Hexane 2. Hexane & ethyl acetate.

i) Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentrations (MFC) value's of bioactive component of *A. angusta* St. extracts against target organisms.

MIC for *A. angusta* St. extracts.

Target organism	MIC/ MFC (µg/ml)
Aspergillus niger	3
Fusarium moniliformae	1
F. oxysporum	6
Rhizopus stolonifer	3

MIC for antibiotic, nystatin.

Target organism	MIC/ MFC (µg/ml)
Aspergillus niger	9
Fusarium moniliformae	9
F. oxysporum	7
Rhizopus stolonifer	6

Activity was performed by using the two fold dilution technique.
Data values represent average of three replicates.

Susceptibility of microorganisms to fresh and stored *A. angusta* St. fresh extract showed 12 mm and stored extract showed 11 mm zone inhibition against *A. niger*. At low or high pH level, 11 mm; at various temperatures (30-60°C), 11 mm and by detergents and enzymes treatments, 11mm and 12 mm zone inhibitions are observed. The bioactive components were stable for a period of 3 months at 4°C. Overall the inhibitions are stable. Vegetative stage of plant extracts has 0.179 and reproductive stage of plant extracts has 0.191 Rf value. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) value's of bioactive components of extracts against target organisms varies from 2 to 6 µg/ml.

B) *Plagiochasma simulensis* Kash.

a) Susceptibility of microorganisms to fresh and stored *P. simulensis* Kash. extracts.

Susceptibility of extracts was evaluated and is documented in bellow -

Susceptibility of test organisms to fresh and stored *P. simulensis* Kash. extract.

Nature of extract	Inhibition zone (mm) against target organisms			
	A	B	C	D
Fresh extract	12	NI	13	NI
Stored extract	11	NI	13	NI

A- *Aspergillus niger*, B- *Fusarium moniliformae*,
C - *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

b) Effect of pH on activity and stability of bioactive compounds from *P. simulensis* Kash. extracts.

Stability of bioactive compounds from extract, at different pH was checked by pre-incubating for 1 hr. in phosphate buffer at different pH ranging from 5.7 to 8.0. The bioactive extract was quite stable within this pH range as tested against target organisms such as *A. niger*, *F. moniliformae*, *F. oxysporum*, and *R. stolonifer*. After incubation of bioactive extracts at pH in range of 5.7 to 8.0, the maximum residual activity was observed at pH range 6.8-8.0, where as the activity decreased at pH 6.8 and bellow.

Effect of pH on activity of bioactive components from *P. simulensis* Kash. extracts.

pH	Inhibition zone (mm) against target organisms			
	A	B	C	D
5.7	9	NI	11	NI
6.8	11	NI	12	NI
7.3	11	NI	12	NI
8.0	11	NI	12	NI

A-*Aspergillus niger*, B- *Fusarium moniliformae*,
C- *F. oxysporum*, D- *Rhizopus stolonifer*.

c) Thermal stability of bioactive components from *P. simulensis* Kash. extracts.

Since the activity of bioactive extracts was quite stable at 4°C, experiments are also conducted to see the effect of elevated temperature on stability of bioactive compounds. For this purpose, the bioactive extracts was kept at various temperature (30-60°C) for one hour. Bioactive compounds was stable at different temperatures.

Thermal stability of bioactive compounds from *P. simulensis* Kash. extracts.

Temperature (°C)	Inhibition zone (in mm) against target organisms.	
	A	C
30	11	13
40	11	13
50	11	12
60	10	12

NI : No Inhibition, A- *Aspergillus niger*, C - *Fusarium oxysporum*

Data values represent average of three replicates.

d) Effect of detergents on bioactivity of *P. simulensis* Kash. extracts against target organisms.

Effect of various detergents *viz.* Tween-20 and cetrimide on activity of bioactive components from extracts was determined by mixing them with extracts, and incubating for 6 hrs at 30°C. The activity was checked against target organisms. Detergents added to distilled water were used as control to check the effect of detergents themselves on *F. oxysporum*. The bioactive compounds alone showed 12 mm inhibition zone diameter against *F. oxysporum*, while inhibition zone diameter obtained with mixer of bioactive extract and detergents. This clearly indicated that, there was no significant loss of antifungal activity of bioactive compounds after treatment with detergents.

Effect of detergents on antibiotic activity of *P. simulensis* Kash. extracts against target organisms.

Detergent	Inhibition zone (mm) against target organisms.	
	Without bioactive extracts	With bioactive extracts
	A	C
Tween-20	11	12
Cetrimide	10	12

A- *Aspergillus niger*, C - *Fusarium oxysporum*
The bioactive extracts concentration 100 µl/ml.
Data values represent average of three replicates.

e) Effect of enzymes on activity of bioactive components from *P. simulensis* Kash. extracts against target organisms.

Sensitivity of extracts bioactive components to enzymes like Protease K, and Lipase was evaluated. The bioactive compounds of extract was mixed with respective enzymes and incubated for 3 hrs. and activity of bioactive compounds was assayed against *A. niger* and *F. oxysporum*. The bioactive components alone showed 11 mm and 12 mm inhibition zone diameter against *A. niger* and *F. oxysporum*, while inhibition zone diameter obtained with mixture of bioactive extracts and enzyme ranged between 10 to 11 mm in case of *A. niger*, and 11 to 13 mm in case of *F. oxysporum*. This clearly indicated that, there was no significant loss of antifungal activity of bioactive extracts after treatment with enzymes.

Effect of enzymes on the activity of bioactive components from *P. simulensis* Kash. extracts against target organisms.

Test	Inhibition zone (mm) against target organisms	
	A	C
Protease K + bioactive extract	11	12
Lipase + bioactive extract	11	12
Antibiotics	12	11

A- *Aspergillus niger*, C - *Fusarium oxysporum*.
Data values represent average of three replicates.

f) Solubility of bioactive components from *P. simulensis* Kash. extracts.

The liverwort *P. simulensis* Kash. Dry extract powder is soluble in each organic solvent *viz.* acetone, ethanol, petroleum ether, and distilled water.

g) Determination of shelf life of bioactive compounds from *P. simulensis* Kash. extracts.

To determine the shelf life of bioactive components from extracts. The extract was stored at 4°C in different ampoules for 1 week, 1 month, 2 month, 3 month. After the specified storage period, antifungal activity of the extract was determined by 'disc diffusion assay' method. The bioactive components were stable for a period of 3 months at 4°C.

Effect of shelf life of bioactive components from *P. simulensis* Kash. extracts against target organisms.

Incubation time	Inhibition zone (mm) against target organisms			
	A	B	C	D
1 week	11.0	NI	13.0	NI
1 month	11.0	NI	12.5	NI
2 month	11.0	NI	12.0	NI
3 month	10.0	NI	12.0	NI

A- *Aspergillus niger*, B- *A. moniliformae*,
C - *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

h) Thin Layer Chromatography (TLC) of bioactive compounds from *P. simulensis* Kash. extracts.

Majority of the extracts resolved best with hexane & ethyl acetate, and pure hexane. *P. simulensis* Kash. vegetative stage of plant extracts has no any clear resolved component but, reproductive stage of plant extracts resolved single component.

TLC results of acetone extracts of *P. simulensis* Kash. (Veg. stage).

Species	Solvent system	No. of components	Rf value
P. simulensis Kash.	2	NR	NR

NR : Not reported.

TLC results of acetone extracts of *P. simulensis* Kash. (Reproductive stage).

Species	Solvent system	No. of components	Rf value
P. simulensis Kash.	2	1	0.112

Solvent systems - 1. Hexane 2. Hexane & ethyl acetate

i) Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) value's of bioactive components of *P. simulensis* Kash. extracts against different target organisms.

MIC for *P. simulensis* Kash. extracts.

Target organism	MIC/ MFC (µg/ml)
Aspergillus niger	4
Fusarium moniliformae	2
F. oxysporum	5
Rhizopus stolonifer	2

MIC for antibiotic, nystatin.

Target organism	MIC/ MFC (µg/ml)
Aspergillus niger	10
Fusarium moniliformae	9
F. oxysporum	10
Rhizopus stolonifer	Nil

Activity was performed by using the two fold dilution technique.
Data values represent average of three replicates.

Plagiochasma simulensis Kash. fresh extracts showed 12 mm, and stored extract showed 11 mm zone inhibition against *A. niger*. At low or high pH level, 9 mm to 11 mm zone inhibitions; at various temperatures (30-60°C), 11 mm; by detergents and enzymes treatments, 10 mm and 11 mm inhibitions observed. The bioactive components were stable for a period of 3 months at 4°C. Overall the inhibitions are stable. Vegetative stage of plant extracts has no Rf value but, reproductive stage of plant extracts indicates 0.112 Rf value. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) value's of bioactive component of extracts against target organisms varies from 2 to 5 µg/ml.

C). *Targionia hypophylla* (Mich.) L.

a) Susceptibility of microorganisms to fresh and stored *T. hypophylla* (Mich.) L. extracts.

Susceptibility of extracts was evaluated and is documented bellow -

Susceptibility of test organisms to fresh and stored *T. hypophylla* (Mich.) L. extracts.

Nature of extract	Inhibition zone (mm) against target organisms			
	A	B	C	D
Fresh extract	8	NI	10	NI
Stored extract	7	NI	10	NI

NI : No Inhibition.

A- *Aspergillus niger*, B- *Fusarium moniliformae*,
C - *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

b) Effect of pH on activity and stability of bioactive components from *T. hypophylla* (Mich.) L. extracts.

Stability of bioactive components from extract, at different pH was checked by pre-incubating for 1 hr. in phosphate buffer at different pH ranging from 5.7 to 8.0. The bioactive extract was quite stable within this pH range as tested against target organisms such as *A. niger*, *F. moniliformae*, *F. oxysporum*, and *R. stolonifer*. After incubation of bioactive extracts at pH in range of 5.7 to 8.0, the maximum residual activity was observed at pH range of 6.8 to 8.0, where as the activity decreased at pH 6.8 and bellow.

Effect of pH on activity of bioactive extracts from *T. hypophylla* (Mich.) L. extracts.

pH	Inhibition zone (mm) against target organisms.			
	A	B	C	D
5.7	7	NI	9	NI
6.8	8	NI	10	NI
7.3	7	NI	10	NI
8.0	8	NI	10	NI

A- *Aspergillus niger*, B- *Fusarium moniliformae*,
C - *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

c) Thermal stability of bioactive components from *T. hypophylla* (Mich.) L. extracts.

Since the activity of bioactive extracts was quite stable at 4°C, experiments were also conducted to see the effect of elevated temperature on stability of bioactive compounds. For this purpose, the bioactive extracts was kept at various temperature (30-60°C) for one hour. The bioactive compounds was stable at different temperatures.

Thermal stability of the bioactive extracts from *T. hypophylla* (Mich.) L. extracts.

Temperature (°C)	Inhibition zone diameter (in mm) against target organisms	
	A	C
30	8	10
40	7	9
50	7	9
60	7	9

A- *Aspergillus niger*, C - *Fusarium oxysporum*
Data values represent average of three replicates.

d) Effect of detergents on bioactivity of *T. hypophylla* (Mich.) L. extracts against target organisms .

Effect of various detergents *viz*. Tween-20 and cetrimide on activity of bioactive components from extracts was determined by mixing them with extracts, and incubating for 6 hrs. at 30°C. The activity was checked against target organisms. Detergents added to distilled water were used as control to check the effect of detergents themselves on *F. oxysporum*. The bioactive components alone showed 10 mm inhibition zone diameter against *F. oxysporum*, while inhibition zone diameter obtained with mixer of bioactive extract and detergents. This clearly indicated that, there was no significant loss of antifungal activity of bioactive compounds after treatment with detergents.

Effect of detergents on antibiotic activity of *T. hypophylla* (Mich.) L. extracts against target organisms.

Detergent	Inhibition zone diameter (mm) against target organisms	
	Without bioactive extracts	With bioactive extracts
	A	C
Tween-20	7	9
Cetrimide	8	10

A- *Aspergillus niger*, C - *Fusarium oxysporum*
The bioactive extracts concentration 100 µl/ml.
Data values represent average of three replicates.

e) Effect of enzymes on activity of bioactive compounds from *T. hypophylla* (Mich.) L. extracts against target organisms.

Sensitivity of extracts bioactive components to enzymes like Protease K, and Lipase was evaluated. The bioactive compounds of extract was mixed with respective enzymes and incubated for 3 hrs. and activity of bioactive compounds was assayed against *A. niger* and *F. oxysporum*. The antibiotic compound alone showed 7 mm and 10 mm inhibition zone diameter against *A. niger* and *F. oxysporum*, while inhibition zone

diameter obtained with mixture of bioactive extracts and enzyme ranged between 7 to 8 mm in case of *A. niger*, and 9 to 10 mm in case of *F. oxysporum*. This clearly indicated that, there was no significant loss of antifungal activity of bioactive components after treatment with enzymes.

Effect of enzymes on the activity of bioactive components from *T. hypophylla* (Mich.) L. extracts against target organisms.

Test	Inhibition zone (mm) against target organisms	
	A	C
Protease K + bioactive extract	8	10
Lipase + bioactive extract	7	10
Antibiotics	11	11

A- *Aspergillus niger*, C - *Fusarium oxysporum*.
Data values represent average of three replicates.

f) Solubility of bioactive components from *T. hypophylla* (Mich.) L. extracts.

The liverwort *T. hypophylla* (Mich.) L. dry extract powder is soluble in each organic solvent *viz.* acetone, ethanol, petroleum ether, and distilled water.

g) Determination of shelf life of bioactive compounds from *T. hypophylla* (Mich.) L. extracts.

To determine the shelf life of bioactive components from extracts. Extract was stored at 4°C in different ampoules for 1 week, 1 month, 2 month, 3 month. After the specified storage period, antifungal activity of the extract was determined by 'disc diffusion assay' method. The bioactive components were stable for a period of 3 months at 4°C.

Effect of shelf life of bioactive components from *T. hypophylla* (Mich.) L. extracts against target organisms.

Incubation time	Inhibition zone (mm) against target organisms			
	A	B	C	D
1 week	8	-	10	-
1 month	8	-	9	-
2 month	7	-	9	-
3 month	7	-	9	-

A- *Aspergillus niger*, B- *A. moniliformae*,
C - *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

h) Thin Layer Chromatography (TLC) of bioactive compounds from *T. hypophylla* (Mich.) L. extracts.

T. hypophylla (Mich.) L. gives good separation with pure hexane, and hexane and ethyl acetate. Maximum number of two components were resolved.

TLC results of acetone extracts of *T. hypophylla* (Mich.) L. (Veg. stage).

Species	Solvent system	No. of components	Rf value
T. hypophylla (Mich.) L.	1	2	0.138; 0.132

TLC results of acetone extracts of *T. hypophylla* (Mich.) L. (Reproductive stage).

Species	Solvent system	No. of components	Rf value
T. hypophylla (Mich.) L.	1	2	0.152; 0.146

Solvent systems - 1. Hexane 2. Hexane & ethyl acetate

i) Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) value's of bioactive component of *T. hypophylla* (Mich.) L. extracts against different target organisms.

MIC for *T. hypophylla* (Mich.) L. extracts.

Target organism	MIC/ MFC (µg/ml)
Aspergillus niger	3
Fusarium moniliformae	1
F. oxysporum	6
Rhizopus stolonifer	3

The MIC for antibiotic, nystatin.

Target organism	MIC/ MFC (µg/ml)
Aspergillus niger	7
Fusarium moniliformae	10
F. oxysporum	10
Rhizopus stolonifer	9

Activity was performed by using the two fold dilution technique.
Data values represent average of three replicates.

Targionia hypophylla (Mich.) L. fresh extract showed 8 mm and stored extract showed 7 mm zone inhibition against *A. niger*. At low or high pH level, 7 mm to 8 mm; at various temperatures (30-60°C), 8 mm; by detergents and enzymes treatments, 7 mm to 8 mm inhibitions. The bioactive components were stable for a period of 3 months at 4°C. Vegetative stage of plant extract has 0.138 and 0.132 Rf value's whereas, reproductive stage of plant extracts has 0.152; 0.146 Rf value's. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) value's of bioactive component of extracts against target organisms varies from 1 to 6 µg/ml.

D) *Cyathodium tuberosum* Kash.

a) Susceptibility of microorganisms to fresh and stored *C. tuberosum* Kash. extracts.

Susceptibility of extracts was evaluated and is documented bellow -

Susceptibility of test organisms to fresh and stored *C. tuberosum* Kash. extracts.

Nature of extract	Inhibition zone (mm) against target organisms			
	A	B	C	D
Fresh extract	11	NI	9	NI
Stored extract	11	NI	8	NI

NI : No Inhibition.

A- *Aspergillus niger*, B- *Fusarium moniliformae*,
C- *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Each data point represents average of three replicates.

b) Effect of pH on activity and stability of bioactive compounds from *C. tuberosum* Kash. extracts.

Stability of bioactive compounds from extract, at different pH was checked by pre-incubating for 1 hr. in phosphate buffer at different pH ranging from 5.7 to 8.0. The bioactive extract was quite stable within this pH range as tested against target organisms such as *A. niger*, *F. moniliformae*, *F. oxysporum*, and *R. stolonifer*. After incubation of bioactive extracts at pH in range of 5.7 to 8.0, the maximum residual activity was observed at pH range of 6.8 to 8.0, where as the activity decreased at pH 6.8 and bellow.

Effect of pH on activity of bioactive extracts from *C. tuberosum* Kash. extracts.

pH	Inhibition zone (mm) against target organisms.			
	A	B	C	D
5.7	8	NI	8	NI
6.8	10	NI	9	NI
7.3	9	NI	9	NI
8.0	10	NI	9	NI

A- *Aspergillus niger*, B- *Fusarium moniliformae*,
C - *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

c) Thermal stability of bioactive components from *C. tuberosum* Kash. extracts.

Since the activity of bioactive extracts was quite stable at 4°C, experiments are also conducted to see the effect of elevated temperature on stability of bioactive compounds. For this purpose, the bioactive extracts was kept at various temperature (30-60°C) for one hour. The bioactive compounds was stable at different temperatures.

Thermal stability of the bioactive compounds from *C. tuberosum* Kash. extracts.

Temperature (°C)	Inhibition zone (mm) against target organisms	
	A	C
30	11	9
40	11	8
50	10	8
60	10	8

A- *Aspergillus niger*, C - *Fusarium oxysporum*
Data values represent average of three replicates.

d) Effect of detergents on bioactivity of *C. tuberosum* Kash. extracts against target organisms.

Effect of various detergents *viz*. Tween-20 and cetrimide on activity of bioactive components from extracts was determined by mixing them with extracts, and incubating for 6 hrs at 30°C. The activity was checked against target organisms. Detergents added to distilled water were used as control to check the effect of detergents themselves on *F. oxysporum*. The bioactive compounds alone showed 8 mm inhibition zone diameter against *F. oxysporum*, while inhibition zone diameter obtained with mixer of bioactive extract and detergents. This clearly indicated that, there was no significant loss of antifungal activity of bioactive compounds after treatment with detergents.

Effect of detergents on antibiotic activity of *C. tuberosum* Kash. extracts against target organisms.

Detergent	Inhibition zone (mm) against target organisms.	
	Without bioactive extracts	With bioactive extracts
	A	C
Tween-20	11	9
Cetrimide	11	8

A- *Aspergillus niger*, C - *Fusarium oxysporum*

The bioactive extracts concentration 100 µl/ml.

Data values represent average of three replicates.

e) Effect of enzymes on activity of bioactive compounds from *C. tuberosum* Kash. extracts against target organisms.

Sensitivity of extracts bioactive components to enzymes like Protease K, and Lipase was evaluated. The bioactive compounds of extract was mixed with respective enzymes and incubated for 3 hrs, and activity of bioactive compounds was assayed against *A. niger* and *F. oxysporum*. The antibiotic compound alone showed 11 mm and

8 mm inhibition zone diameter against *A. niger* and *F. oxysporum*, while inhibition zone diameter obtained with mixture of bioactive extracts and enzyme ranged between 10 to 11 mm in case of *A. niger*, and 8 to 9 mm in case of *F. oxysporum*. This clearly indicated that there was no significant loss of antifungal activity of the bioactive extract after treatment with enzymes.

Effect of enzymes on the activity of bioactive components from *C. tuberosum* Kash. extracts against target organisms.

Test	Inhibition zone (mm) against target organisms	
	A	C
Protease K + bioactive extract	10.5	8.0
Lipase + bioactive extract	11.0	9.0
Antibiotics	11.0	8.0

A- *Aspergillus niger*, C - *Fusarium oxysporum*
Data values represent average of three replicates.

f) Solubility of bioactive components from *C. tuberosum* Kash. extracts.

C. tuberosum Kash. dry extract powder is soluble in each organic solvent *viz.* acetone, ethanol, petroleum ether, and distilled water.

g) Determination of shelf life of bioactive compounds from *C. tuberosum* Kash. extracts.

To determine the shelf life of bioactive components from extracts. The extract was stored at 4°C in different ampoules for 1 week, 1 month, 2 month, 3 month. After the specified storage period, the antifungal activity of the extract was determined by 'disc diffusion assay' method. The bioactive components were stable for a period of 3 months at 4°C.

Effect of shelf life of bioactive components from *C. tuberosum* Kash. extracts against target organisms.

Incubation time	Inhibition zone (mm) against target organisms.			
	A	B	C	D
1 week	11.5	NI	8.0	NI
1 month	11.0	NI	8.0	NI
2 month	11.0	NI	9.0	NI
3 month	10.0	NI	9.0	NI

A- *Aspergillus niger*, B- *A. moniliformae*,
C - *Fusarium oxysporum*, D- *Rhizopus stolonifer*
Data values represent average of three replicates.

h) Thin Layer Chromatography (TLC) of bioactive compounds from *C. tuberosum* Kash. extracts.

C. tuberosum Kash. extract resolved better in pure hexane. Maximum number of two components are resolved.

TLC results of acetone extracts of *C. tuberosum* Kash. (Veg. stage).

Species	Solvent system	No. of components	Rf value
C. tuberosum Kash.	1	2	0.118; 0.112

Solvent systems - 1. Hexane 2. Hexane & ethyl acetate.

TLC results of acetone extracts of *C. tuberosum* Kash. (Reprod. stage).

Species	Solvent system	No. of components	Rf value
C. tuberosum Kash.	1	2	0.133; 0.122

Solvent systems - 1. Hexane 2. Hexane & ethyl acetate.

i) Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) value's of bioactive components of *C. tuberosum* Kash. extracts against different target organisms.

MIC for *C. tuberosum* Kash. extracts.

Target organism	MIC/ MFC (µg/ml)
Aspergillus niger	5
Fusarium moniliformae	Nil
F. oxysporum	5
Rhizopus stolonifer	7

MIC for antibiotic, nystatin.

Target organism	MIC/ MFC (µg/ml)
Aspergillus niger	8
Fusarium moniliformae	Nil
F. oxysporum	10
Rhizopus stolonifer	8

Activity was performed by using the two fold dilution technique.
Data values represent average of three replicates.

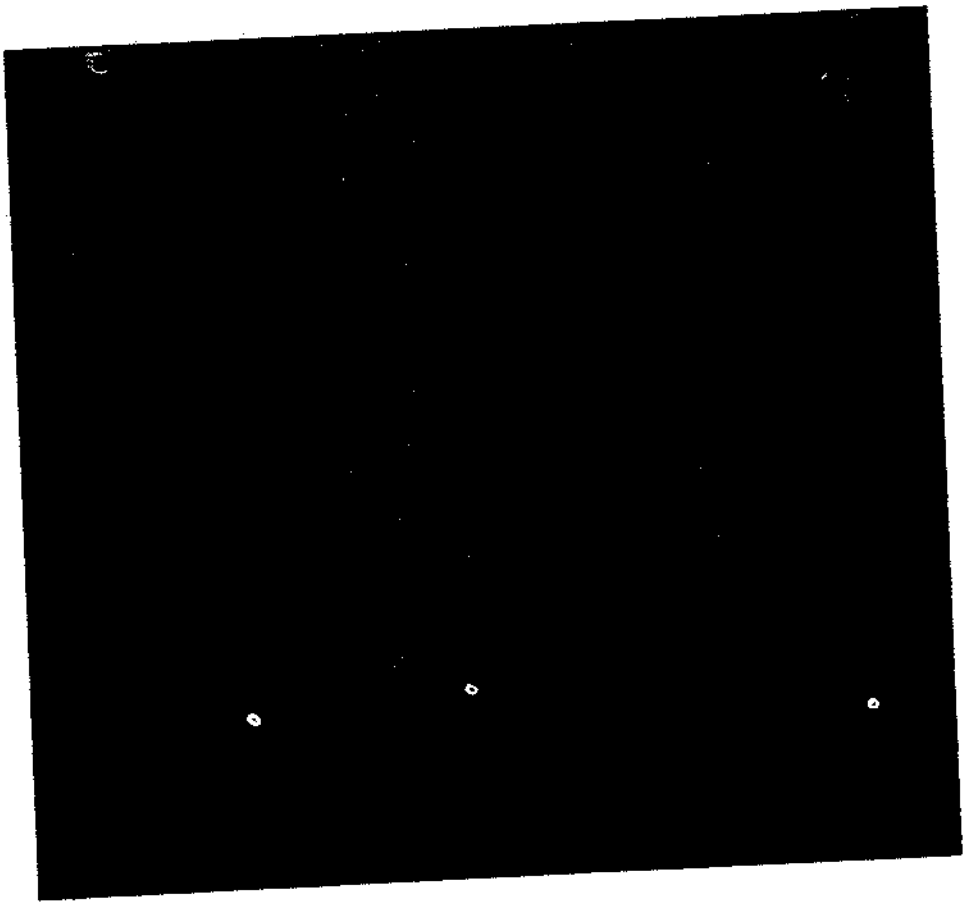
Cyathodium tuberosum Kash. fresh extract showed 11 mm, and stored extract showed 11 mm zone inhibition against *Aspergillus niger*. At low or high pH level, 8 mm to 10 mm inhibitions; at various temperatures (30- 60°C), 11 mm, and by detergents and enzymes treatments, both inhibitions are same (11 mm). The bioactive components were stable for a period of 3 months at 4°C. Overall inhibitions are stable. Vegetative stage of plant extracts has 0.118 and 0.112 Rf value's and reproductive plant extracts has 0.133, 0.122 Rf value's. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) value's of bioactive component of extracts against target organisms varies from 1 to 6 µg/ml.

These all Rf value's of selected thalloid liverworts extracts determines the class of compounds specially steroids and terpenes. Extracts containing volatile oils, steroids etc. evaporates quickly therefore mark on plate where reagent colour indicated. According to chromatography (TLC), suggesting that the majority of secondary metabolites were stable, over this time period. Bioactive extracts of selected thalloid liverworts were characterized for susceptibility of target organisms to fresh and stored extracts, effect of pH, temperature, detergents, enzymes, shelf life, solubility of bioactive components, Thin Layer Chromatography (TLC), and Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) value's determination. Antifungal compounds were isolated from the New Zealand liverwort *Plagiochilla faciculata* (Lorimer and Perry, 1994).

The most active hexane fraction was subjected to chemical analysis to determine the classes of compounds present in it (Wagner *et al.*, 1984). Minimum Fungicidal Concentrations (MFC) value's were determined as the lowest concentration that prevented the growth of subculture (Lavermicocca *et al.* 2003). The most active hexane fractions was subjected to chemical analysis. The fractions was subjected to silica gel Thin Layer Chromatography (TLC) using chloroform as a solvent. The chromatograms

were sprayed with various reagents to detect the presence of various classes of compounds by exposing to UV fluroscence and then inspected (Subhisha and Subramoniam, 2005). The fresh plant material (0.85g) was extracted with acetone and make crude extract concentration (61 mg), then crude acetone extract was chromatographed on Sephadex LH-20 eluting with ethyl acetate: hexane (1:1); petroleum ether & ethyl acetate (1:1) step gradient, yielded one fraction which subjected to TLC (Xiaowei, 2007). In present work the crude acetone extracts of selected thalloids eluted with ethyl acetate: hexane (1:1) and pure hexane, step gradient fractions. MIC and MFC value's for the chloroform extract of *Thuidium delicatulum* used against organisms reported (Ergin and Barbaros, 2009).

6. SUMMARY AND CONCLUSIONS



SUMMARY AND CONCLUSIONS

The thesis mainly comprises the studies on the antimicrobial properties of thalloid liverworts from Western Ghats of Maharashtra. Chapter one gives information about the floristic work done in India by different scientists workers starting from the monumental work by Kashyap, (1919). It also explains the need of work done to be carried out in Western Ghats of Maharashtra. It describes the forces which promoted the authors to carry out this research work.

Chapter two deals with review of literature with reference to importance of bryophytes in India, specially at Western Ghats of Maharashtra, chemosystematics of liverworts, antibiotic compounds and their properties in bryophytes specially noted in liverworts.

Chapter three explains the material and methods of the work carried out during the course of investigation with the survey of bryophytes undertaken at ecologically different altitudinal areas of Western Ghats of Maharashtra (Map. II). It included the collection, storage and identification of thalloid liverworts during July, 2008 to September, 2011.

For purpose of analysis of physico-chemical and biological characteristics, soil samples are separated from thalloid liverworts. Elements analysis is made at Soil Testing Laboratory, Someshwarnager, Krushi Vidnyan Kendra, Shardanagar and in Post Graduate Research Centre, Department of Botany, Tuljaram Chaturchand College, Baramati, Dist. Pune.

The chapter also describes the antimicrobial screening of 11 thalloid liverworts, method of extractions made with different organic solvents and distilled water. The thalloid liverworts are as *Fossombronia indica* St., *Sewardiella tuberifera* Kash., *Exormotheca tuberifera* Kash., *Asterella angusta* St., *Plagiochasma articulatum* Kash.,

P. appendiculatum L. et L., *P. simulensis* Kash., *Targionia hypophylla* (Mich.) L., *Cyathodium tuberosum* Kash., *Riccia discolar* L.et L. and *R. fluitans* L. The plant extracts are subjected for antibacterial and antifungal activity through 'disc diffusion assay' method. The cultures are obtained from National Collection of Industrial Microorganisms (NICM), Biochemical Sciences Division, National Chemical Laboratory, Pune, India. It included four bacterial strains *viz.*, *Bacillus substilis* (NCIM 2697), *Escherichia coli* (NCIM 2067), *Pseudomonas aeruginosa* (NCIM 2200), *Staphylococcus aureus* (NCIM 2492) and four fungal strains *viz.*, *Aspergillus niger* (NCIM 507), *Fusarium moniliformae* (NCIM 1276), *Fusarium oxysporum* (NCIM 1072), and *Rhizopus stolonifer* (NCIM 1139).

Chapter four introduces the characterization of selected thalloid liverworts extracts for antimicrobial properties. Bioactive fresh and stored extracts extracts are characterized for susceptibility of target organisms. The extracts are subjected for analysis of effect of pH, temperature, detergents, enzymes, shelf life, solubility of bioactive components, Thin Layer Chromatography (TLC), Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC).

In chapter five, results and discussion is major part of the work. It gives detailed information about identified specimens and verification through comparison with the preserved material, in wet Herbarium voucher specimens stored at Laboratory. The occurrence of 11 species of thalloid liverworts belonging to 08 genera, distributed over 05 families are identified are used for physico-chemical, biological and antimicrobial screening. The collected taxa belonging to the class hepaticae are arranged systematically according to the classification suggested by Schuster (1958, 1979). This chapter provides the key for identification of liverworts genera during our field tours.

In this chapter, the physical properties like soil colour, texture, structure, soil constitution, new growths, electric conductivity and pH as well as soil colour, structure,

texture, soil constitution, new growth from each localities are documented. Only at Panhala locality, some soil samples with lime and silica indicated new growth formation. Sinhagad soil showed iron oxides with silica and all soil samples from remaining localities are without constitution. The chemical characteristics analysis made for available nitrogen (N), phosphorous (P) and potassium (K); sodium (Na), calcium (Ca) ion salts and organic carbon percentage (C%). Soil samples are categorized as per localities from basal, middle and high altitudinal areas. The fungal isolation is made by serial dilution method, and identifications are done by using standard literature.

Available 'N' contents are high 561.94 Kg/ha in associated soil of *Targionia hypophylla* Kash., whereas minimum in *Cyathodium tuberosum* Kash. Available organic carbon contents high (3.31 %) in *Cyathodium tuberosum* Kash. at Rajgad (5- C, Table No. 4). High carbon contents in soils of *Plagiochasma simulensis* Kash., whereas, minimum in *Fossombronia indica* St. (188.16%) at Purandar. Available N (183 Kg/ha) and organic carbon percent (0.76 %) of *Exormotheca tuberifera* Kash. soil is high at Sinhagad (Table No. 5). The available N (253 Kg/ha) and the available organic carbon percent (1.05 %) of *Plagiochasma simulensis* Kash. soil is high at Rayereshwar. At Kas;Satara region the available 'N' concentration more (186.03 Kg/ha) in *Cyatodium tuberosum* Kash. as well as (255.37 Kg/ha) in *Asterella angusta* St. at Lonawala region (Table No. 6). At Panhala regions areas 'C' (3.45%) in *Cyathodium tuberosum* Kash. associated soil and 'N' in *Plagiochasma articulatum* Kash. soil (226 Kg/ha) is more observed (Table No. 7).

Antimicrobial screening of *Fossombronia indica* St. extracts indicates the greater inhibitory activity by ethanol extract against *S. aureus* at Purandar (5- E, Table No. 2). *Fusarium oxysporum* is very sensitive to *Sewardiella tuberifera* Kash. ethanol extracts. Only *A. niger* showed sensitivity to organic solvents and aqueous extracts of *Exormotheca tuberifera* Kash. at Purandar and Khandala (5- E, Table No. 5). *A. angusta*

St. ethanolic extracts from Lonawala showed more antifungal activity, followed by acetone, petroleum ether and aqueous extracts (5- E, Table No. 7). Three different species of genus *Plagiochasma* L. from Khandala, Kas;Satara and Panhala localities *viz.*, *P. articulatum* Kash., *P. appendiculatum* L. and *P. simulensis* Kash. are screened for antimicrobial activities (5- E, Table Nos. 10 to 14). More antibacterial activity are exhibited by acetone extract of *P. articulatum* Kash. against *S. aureus* and lower activity against *B. substilis*. Acetone extract exhibits greater antifungal activity against *A. niger* whereas, minimum activity against *F. moniliformae* and *F. oxysporum*. In general, acetone and n-butanol extract of *P. simulensis* Kash. is more effective as compare to aqueous and methanol extracts at Sinhagad (5- E, Table Nos. 13 to 14). *T. hypophylla* Kash. petroleum ether extract was shown the greater inhibitory activity against *F. oxysporum*, and *E. coli*. *Cyathodium tuberosum* Kash. ethanol extract has greater antimicrobial activity against *A. niger* and *P. aureginosa*; followed by activity of acetone extract against *F. moniliformae* and *E. coli*. *Riccia discolor* L. from Purandar. showed the greater antifungal activity against *A. niger*. *R. fluitans* L. acetone extract exhibits greater antifungal activity against *F. oxysporum* at Kas; Satara region.

Characterization of selected thalloid liverworts extracts for antimicrobial properties and their evaluation, documentation, results and discussion is mentioned. Susceptibility of microorganisms to fresh and stored *A. angusta* St. fresh extract showed 12 mm and stored extract showed 11 mm zone inhibition against *A. niger*. At low or high pH level zone inhibition is 11 mm; at various temperatures (30 to 60°C), 11 mm inhibition and by detergents and enzymes treatments, 11mm and 12 mm zone inhibitions, respectively. The bioactive components were stable for a period of 3 months at 4°C. Over all the inhibitions are stable. Vegetative stage of plant extracts has 0.179 and reproductive stage of plant extracts has 0.191 Rf value. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values of bioactive components of extracts against target organisms varies from 2 to 6 µg/ml.

P. simulensis Kash. fresh extracts showed 12 mm, and stored extract showed 11 mm zone inhibition against *A. niger*. At low or high pH level, 9 mm to 11 mm zone inhibitions; at various temperatures (30 to 60°C), 11 mm; by detergents and enzymes treatments, 10 mm and 11 mm inhibitions observed. The bioactive components have been found stable for a period of 3 months at 4°C. Over all the inhibitions are stable. Vegetative stage of plant extracts has no bioactive constituents reported, but, reproductive stage of plant extracts indicates 0.112 Rf value. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values of bioactive component of extracts against target organisms varies from 2 to 5 µg/ml.

T. hypophylla (Mich.) L. fresh extract showed 8 mm and stored extract showed 7 mm zone inhibition against *A. niger*. At low or high pH level, 7 mm to 8 mm inhibitions; at various temperatures (30 to 60°C) zone of inhibition is 8 mm. The effect of detergents and enzymes treatments showed 7 to 8 mm inhibitions. The bioactive components were stable for a period of 3 months at 4°C. The maximum number of two components are resolved. Vegetative stage of plant extract has 0.138 and 0.132 Rf value's whereas, reproductive stage of plant extracts has 0.152; 0.146 Rf values. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values of bioactive component of extracts against target organisms varies from 1 to 6 µg/ml.

C. tuberosum Kash. fresh extract showed 11 mm zone of inhibition and stored extract showed 11 mm inhibition against *A. niger*. At low or high pH level, 8 mm to 10 mm inhibitions; at various temperatures (30 to 60°C), 11 mm, and by detergents and enzymes treatments, both inhibitions are same (11 mm). The bioactive components were stable for a period of 3 months at 4°C. Over all inhibitions are stable. Vegetative stage of plant extracts has 0.118 and 0.112 Rf values and reproductive plant extracts has 0.133, 0.122 Rf values. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal

Concentration (MFC) values of bioactive component of extracts against target organisms varies from 1 to 6 µg/ml. According to this we summarize the findings that -

- Soil samples from 13 different localities as per basal, middle and high altitudinal areas are studied for the analysis.
- There is a significant variation in physical and chemical characteristics of soil associated with liverworts.
- The soil analysis of different localities earlier study has shown a general trend of high K values and low Ca values. Obviously, high K values are promoting fast microbial growth in the soil.
- Low Ca i.e. Ca deficiency may be one of the reason for susceptibility of organisms towards the microbial pathogenesity, resulting in synthesis of more bioactive molecules from thalloid liverworts towards resistance mechanism.
- The rich organic matter (C%) and sufficient amount of available 'N' content favours the growth of thalloid liverworts.
- General pH range of soil at different localities is 5 to 7 pH, which favours the growth of thalloid liverworts (5- C, Table No. 4 to 9).
- *Riccia discolor* is reported to occur in acidic conditions (Table No. 6).
- Organic matter (C %) is rich in soil samples of middle altitudinal areas from Kas;Satara and basal altitudinal areas from Pachgani, whereas values are average in soil of middle areas from Sinhagad and Lonawala (5- C, Table Nos. 5, 6 and 8).
- Electrical conductivity less than 2 $dS.m^{-1}$ is favourable for growth of thalloid liverworts but, E. C. values more than 2 $dS.m^{-1}$ showed unfavourable growth of *Sewardiella tuberifera* Kash. and *Fossombronia indica* St. (5- C, Table Nos. 4 to 9).

- Soil quality is also determined for presence of micro flora (5- D, Table No. 1).
- The fungus *Aspergillus niger* is adapted to different environmental conditions.
- Also, *Aspergillus, Trichoderma, Penicillium* and *Saccharomyces* are dominant genera isolated from soil associated to thalloid liverworts.
- The *Bacillus* and *Pseudomonas* bacteria have been genera reported from different environmental conditions.
- Occurrence of *Trichoderma* in soil samples indicate the disease suppressive characteristic against other organisms.
- *Trichoderma harzianum* and *Saccharomyces cerevisiae* are most beneficial mycoflora observed the luxuriant growth appearance and patches of *Targionia hypophylla* (Mich.) L., *Sewardiella tuberifera* Kash., *Plagiochasma articulatum* Kash. and *P. simulensis* Kash. (Table No. 5 -D).
- There is variation in quality of soil samples because of occurrence of different type of microorganisms.
- Basal altitudinal areas exhibits the greater number of mycoflora than middle and high areas, which also has more humus in soil.
- Association of mycorrhiza *Glomus fasciculatum* with some liverworts like *Plagiochasma simulensis* Kash., *P. articulatum* Kash. and *Asterella angusta* St. are observed, which is beneficial for their luxuriant growth (Table No. 5- D).
- Totally 10 fungal genera are isolated and identified from associated soil of thalloid liverworts.
- Results show that the degree of susceptibility differs between different organisms studied for their antimicrobial activity (5- E, Tables No. 1 to 22).

- Different organic solvent extracts have shown varied levels of antibiotic activity against all the eight test organisms including four fungal and four bacterial strains.
- Thalloid liverworts extracts showed ability to inhibit the fungal spore germination or mycelial growth.
- In majority of the thalloid liverworts, ethanol extracts showed greater sensitivity to tested organisms.
- The extracts had shown strong antimicrobial activity due to presence of bioactive constituents.
- Terrestrial microorganisms release secondary metabolites in the soil and it is are important source of novel natural products having antibiotic activity.
- The tested fungal strains like *Aspergillus niger* and *Fusarium oxysporum* and bacterial strain *Staphylococcus aureus* were more sensitive to ethanol extract, from all screenings.
- *Sewardiella tuberifera* Kash., *Plagiochasma articulatum* Kash extracts from Purandar; *P. simulensis* Kash. extracts from Sinhagad and *Riccia discolor* L. extracts from Rajgad show resistance to the sensitivity of *Rhizopus stolonifer* (5- E, Table Nos. 3, 9, 13 and 20).
- Susceptibility of tested organisms i. e. zone of inhibitions has been proportional to the permeability of cells of the organisms under study.
- The study claims the greater inhibitory activity of *Asterella angusta* St. ethanol extract against *F. oxysporum* (17.5 mm) from Rajgad as well as *P. articulatum* Kash. acetone extract against *S. aureus* (26 mm) from Purandar (5- E, Table No.7 and Table No.10).
- Characterization of selected thalloid liverworts extracts for antimicrobial properties are also studied. This clearly indicated that, there was no significant loss of antibiotic activity of bioactive constituents from extracts

after treatment with enzymes and detergents. There are negligible changes in inhibitory activity by effect of pH, enzymes, detergents, temperature, shelf life for a period of 3 months at 4°C and minimum inhibitory concentration as parameters.

- *Asterella angusta* St. vegetative stage of plant extracts has 0.179 Rf value and reproductive stage of plant extracts has 0.191 Rf value.
- *Plagiochasma simulensis* Kash. vegetative stage of plant extracts has no any clearly resolved component, but reproductive stage of plant extracts showed component with 0.112 Rf value.
- *Targionia hypophylla* (Mich.) L. vegetative stage of plant extract has 0.138 and 0.132 Rf value's and reproductive stage of plant extracts has 0.152; 0.146 Rf values.
- *C. tuberosum* Kash. extract resolved better in pure hexane. Two components are resolved. Vegetative stage of plant extracts has 0.118 and 0.112 Rf values and reproductive plant extracts has 0.133, 0.122 Rf values.
- These all Rf values of selected thalloid liverwort extracts determine the class of compounds, specially steroids and terpenoids (Subhisha and Subramoniam, 2005).
- It is concluded that the antimicrobial properties of thalloid liverworts extracts are due to presence of bioactive constituents.
- This study on the antimicrobial properties of thalloid liverworts and their relation along with physicochemical and biological characteristics of soils from Western Ghats of Maharashtra has been reported first time through this work.

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PUBLICATIONS

List of Research Papers Published in Journals :

1. Kashid, J. K. Kanade, M. B. Telave, A. B. Deokule, S. S. and Chavan, S. J. 2010. Thalloid liverworts and their rhizosphere soil mycoflora from hill forts of Western Ghats, Maharashtra., Geobios, 37 (4): 253-256.
2. Kashid, J. K. Kanade, M. B. Telave, A. B. Deokule, S. S. and Chavan, S. J. 2011. Screening of *Plagiochasma simulensis* Kash. for antifungal activities from Western Ghats of Maharashtra., Advances in Plant Sciences, 24 (1): 73-75.
3. Kashid, J. K. and Chavan, S. J. 2011 Nutrient contents in the rhizosphere of thalloids at Western Ghats, Maharashtra., Bioinfolet, 8 (4): 407-408.

List of Research Papers Published in Proceedings :

1. Kashid, J. K. Kanade, M. B. Telave, A. B. Deokule, S. S. and Chavan, S. J. 2011. Antimicrobial screening and rhizosphere analysis of *Cyathodium tuberosum* Kash. from Western Ghats, Maharashtra- India., UGC sponsored International Conference on 'Biodiversity and its conservation' on 28th-30th January, 2011 organized by P.E.S's Modern College of Arts, Science and Commerce, Shivajinagar, Pune (MH), India.
2. Kanade, M. B. Kashid, J. K. Telave, A. B. Deokule, S. S. and Chavan, S. J. 2011. *Asterella angusta* St., a liverwort screened for antimicrobial activities with nutrient elements analysis from Western Ghats of Maharashtra., UGC sponsored International Conference on 'Biodiversity and its conservation' on 28-30th January, 2011 organized by P.E.S's Modern College of Arts, Science and Commerce, Shivajinagar, Pune (MH), India.
3. Telave, A. B. Kashid, J. K. Kanade, M. B. Deokule, S. S. and Chavan, S. J. 2011. *Targionia hypophylla* L. screened for antimicrobial, physicochemical and biological properties from Western Ghats, Maharashtra- India., UGC sponsored International Conference on 'Biodiversity and its conservation' on 28th-30th

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3. Kashid, J. K. Kashid, L. M. and Chavan, S. J. 2012. Current status of hepatics at regions of Western Ghats, Maharashtra.'' Bioinfolet.
4. Kashid J. K. and Chavan, S. J. 2012. Potential bioactivity of liverwort *Exormotheca tuberifera* Kash. extracts., Geobios.
5. Kashid J. K. and Chavan, S. J. 2013. Fungal Association of Liverworts at Western Ghats of Maharashtra., Adv. Plant Sci., 26 (1).

List of Research Papers Presented in National Conferences:

1. Kashid, J. K., Kanade M. B.,Telave A. B. Deokule S. S. and Chavan, S. J. 2009. Study of mycoflora in rhizosphere of thalloid liverworts from hill forts of Western Ghats, Maharashtra", UGC and University of Pune Sponsored National Conference on 'Recent Trends in Life Sciences' Jointly Organized by P. D. E. A's Annasaheb Magar Mahavidyalaya, Hadapsar, Pune on 18th and 19th Dec. 2009.
2. Kashid, J. K., Kanade M. B., Telave A. B., Deokule S. S. and Chavan, S. J. 2010. Screening of *Plagiochasma simulensis* Kash. for antifungal activities from Western Ghats of Maharashtra, UGC Sponsored National Seminar on "Frontiers

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4. Kashid, J. K., Kanade, M. B., Telave, A. B., Deokule, S. S. and Chavan, S. J. 2011. Studies on the antibacterial activity of *Fossombronia indica* St. extracts and physicochemical, biological analysis of rhizosphere, UGC sponsored National Conference on 'Frontiers in Life Sciences' on 11th-13th December, 2011 organized by Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, Malegaon Bk. Tal. Baramati, Dist. Pune, (MH).

5. Kashid, J. K., Kanade, M. B., Telave, A. B., Deokule, S. S. and Chavan, S. J. 2011. Studies on the genus *Plagiochasma* Lehm. & Lindenb. for physicochemical, biological and antimicrobial properties from Western Ghats of Maharashtra, India., BCUD, University of Pune sponsored, 20th APSI meet 2011 and national Conference on 'Current Innovations in plant sciences' on 5th-7th Feb., 2011 organized by K.T.E's K.J.Somaiya College of Arts, Commerce & Science, Kopargaon, Dist. Ahmednagar.

6. Kashid, J. K., Kanade, M. B., Telave, A. B., Deokule, S. S. and Chavan, S. J. 2011. Antimicrobial, physicochemical and biological properties of *Riccia fluitans* L. from Western Ghats of Maharashtra, India., National Seminar 10th to 12th March 2011, on 'Plant Biodiversity for Sustainable Development' Organized by Department of Botany, University of Pune.

7. Kashid, J. K., Deokule, S. S. and Chavan, S. J. 2012. *Asterella angusta* St. extracts characterization for antimicrobial properties at Western Ghats, Maharashtra., International conference on Current Trends in Medicinal Plants Research, 10-12th January, 2012 Organized by Department of Botany, University of Pune, Pune.

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THALLOID LIVERWORTS AND THEIR RHIZOSPHERE SOIL MYCOFLORA FROM HILL FORTS OF WESTERN GHATS, MAHARASHTRA

J. K. KASHID, M. B. KANADE, A. B. TELAVE, S. S. DEOKULE* and S. J. CHAVAN

Post-Graduate Research Center, Department of Botany, Tuljaram Chaturchand College, Baramati, Dist. Pune - 413 102, India

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ABSTRACT

The present investigation deals with mycoflora in rhizosphere soils of some liverworts from Rajgarh, Purandar, Sinhagarh and Rayreshwar, Western Ghats, Maharashtra. In total 8 fungal genera were identified and their variations occurred as per altitude. *Aspergillus niger, Penicillium notatum* and *Glomus fasciculatum* were most common.

INTRODUCTION

Fungi are often associated with the rhizoids of bryophytes. A large number of bryophytes have the advantages of fungal relationship, providing them with considerably more surface area for nutrients (Ligrone et al., 1993). Liverworts and hornworts are known to form arbuscular mycorrhiza (AM) association (Schuβler, 2000). According to Read et al. (2000) bryophytes have long been known to form association with fungi and a few liverworts endophytes are identified with certainty.

As per studies of Tapwal et al. (2004) mycoflora of rhizosphere influence the growth of bryophytes. Still, little attention was paid to bryophyte-fungal associations. However, the goal of the present study is to find the association of fungi in rhizosphere soils of liverworts from four hill forts of Western Ghats of Maharashtra *viz.* Rajgarh, Purandar, Sinhagarh and Rayreshwar, Dist. Pune.

MATERIALS AND METHODS

The liverworts and their rhizosphere soil samples were collected during August and September, 2009 from four hill forts of Western Ghats. The rhizosphere soils were categorized as per altitude of hill forts and examined. The fungal isolation made by serial dilution method and identifications were done using standard literature (Alexopoulos et al., 2002).

RESULTS AND DISCUSSION

Mycoflora diversity in rhizosphere soils of bryophytes from Western Ghats of Maharashtra is depicted in Table 1. In total 8 fungal genera were identified. The hill fort altitudes (basal, middle and high) seem to affect the frequency and density of mycoflora. Maximum density of fungal species was noticed at basal altitude, but on the contrary high altitude showed lesser number of population.

*Department of Botany, Pune University, Pune - 411 007.

Five fungal genera were identified from 4 liverworts rhizosphere soils at Rajgarh. The rhizosphere soil of *Anthoceros erectus* Kash. from basal altitude showed maximum 4 fungal species viz. *Aspergillus niger*, *Penicillium notatum*, *Saccharomyces cerevisiae* and *Glomus fasciculatum*. *Folioceros khandalensis* Bhardw. and *Cyathodium tuberosum* Kash. showed a single fungus *S. cerevisiae* and *Glomus fasciculatum*, respectively.

Six rhizosphere soils were studied from Purandar and 5 fungal genera were recorded. *Riccia discolor* L. showed *A. niger* and *P. notatum*; *Plagiochasma articulatum* Kash. showed *P. notatum* and *S. cereviseae*; *Plagiochasma simulensis* Kash. showed *Fusarium oxysporum*; *Anthoceros erectus* Kash. showed *Trichoderma harzianum*; *Sewardiella tuberifera* Kash. showed *A. niger*, *S. cerevisiae* and *Fossombronia indica* showed *P. notatum* and *S. cerevisiae*. Among

Table. 1. Mycoflora diversity in rhizosphere soils of thalloid liverworts from Western Ghats of Maharashtra.

Altitude	Genera	A	B	C	D	E	F	G	H
	I-Rajgarh								
Basal	*Anthoceros erectus* Kash.	+	+	+	+	-	-	-	-
Middle	*Targionia hypophylla* (Mich) L.	-	-	+	+	-	-	-	-
High	*Folioceros khandalensis* Bhardw.	-	-	+	-	-	-	-	-
	Cyathodium tuberosum Kash.	-	-	-	-	-	-	-	+
	II-Purandar								
Basal	*Riccia fluitans* L.	+	+	-	-	-	-	-	-
	Plagiochasma articulatum Kash.	-	+	+	-	-	-	-	-
Middle	*Plagiochasma simulensis* Kash.	-	-	-	-	-	+	-	-
	Anthoceros erectus Kash.	-	-	-	-	+	-	-	-
High	*Sewardiella tuberifera* Kash.	+	-	+	-	-	-	-	-
	Fossombronia indica St.	-	+	+	-	-	-	-	-
	III-Sinhagarh								
Basal	*Plagiochasma simulensis* Kash.	+	-	-	+	-	-	-	-
	Sewardiella tuberifera Kash.	-	-	-	-	-	-	-	-
Middle	*Funaria hygrometrica* Hedw.	-	-	-	-	-	-	-	-
	Exermotheca tuberifera Kash.	-	-	-	-	-	-	-	-
High	*Anthoceros erectus* Kash.	-	-	-	-	-	-	-	-
	Plagiochasma articulatum Kash.	-	-	-	+	-	-	-	-
	IV-Rayreshwar								
Basal	*Plagiochasma simulensis* Kash.	-	-	-	-	-	-	+	-
	Plagiochasma articulatum Kash.	-	+	-	-	-	-	-	-
Middle	*Funaria hygrometrica* Hedw.	-	-	-	-	-	-	-	-
	Folioceros khandalensis Bhardw.	-	-	-	-	-	-	-	-
High	*Anthoceros erectus* Kash.	-	-	-	-	-	-	-	-
	Exermotheca tuberifera Kash.	-	-	-	-	-	-	-	-

Present (+), absent (-), A - *Aspergillus niger*, B - *Penicillium notatum*, C - *Saccharomyces cerevisiae*, D - *Glomus fasciculatum*, E - *Trichoderma harzianum*, F - *Fusarium oxysporum*, G - *Mucor mucedo*, and H - *Thielavia basicola*.

these *P. notatum* and *S. cerevisiae* were most common.

A total of 6 rhizosphere soils were screened from Sinhagarh, out of which only *Plagiochasma simulensis* Kash. and *P. articulatum* Kash. showed *A. niger* and *G. fasciculatum*. It is interesting to note that rhizoids of these liverworts showed association of AM fungi.

In six rhizosphere soils from Rayreshwar, only two fungi *P. notatum* and *Mucor mucedo* were recorded with *Plagiochasma simulensis* Kash. and *P. articulatum* Kash.

It has been known for a long time that liverwort and hornworts form AM association. *Glomus claroideum* was investigated for its ability to form AM symbiosis with the hornwort, *Anthoceros punctatus* (L.) (Schuβler, 2000). The AM fungus *Glomus* forming endosymbioses with liverwort *Marchantia foliacea* was reported by Russell & Bulman (2004).

A large number of references are available on mycorrhiza with bryophyte associations. In the present investigation mycorrhiza (AM) *Glomus fasciculatum* in rhizosphere soil of *Anthoceros erectus* Kash., *Targionia hypophylla* (Mich) L., *Plagiochasma simulensis* Kash. and *P. articulatum* Kash. were observed.

Approximately 300 species of Ascomycetes appear to grow as obligates on bryophytes (Doebbeler, 1997). Some fungi, for example *Lamprospora* and *Octospora* are known only from bryophytes (Brouwer, 1999); in other cases, the bryophytes have never been found without fungal association (Doebbeler, 1997). On the contrary, Raspe & De Sloover (1998) suggested that the Discomycetous fungus *Mniaecia jungermanniae*, which lives exclusively on leafy liverworts in the Jungermanniales, might have achieved the first step towards mutualism. This destructive parasite grows inside the bryophyte rhizoids but does not seem to afford any direct benefit to the liverwort that it appears it has a long way to go to reach mutualism.

The symbiotic fungal associations (Ascomycetes and Basidiomycetes) of 28 species, out of 12 families with leafy liverworts (Jungermanniales) were investigated by Ingrid et al. (2003). Lei et al. (2008) noticed an endophytic fungus *Chaetomium fusiformae* from a liverwort, *Scapania verrucosa*. Interesting thing is that *Scapania verrucosa* and its endophyte showed little chemical composition correlation, both of them demonstrated antifungal and antitumour activities. Furthermore, *C. fusiformae* has displayed a wider range of antimicrobial and antitumour activities, which were better than the host plant. These results could support the suggestion of endophytes as an alternative of the host for medicinal activity. There is interesting association of bryophytes and fungi but very little attention has been paid by bryologists in this direction.

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A win-win situation for forests

Forests are a source of livelihood for tens of millions of people around the world. And by drawing down atmospheric carbon dioxide, they also contribute to stabilising greenhouse-gas concentrations. But whether these two types of benefits go hand in hand or conflict with each other remains to be fully understood. To shed more light on this issue, researchers recently analysed data for 80 forests in 10 different countries. They focused on three aspects: forest size, autonomy at the local level to make rules regarding forest management, and ownership (whether by local communities or by national governments). They found that a combination of larger forests and greater local autonomy lead to above-average benefits in terms of livelihood as well as carbon storage. Government ownership was found to result in high livelihood benefits, but this came at a cost to carbon storage. The researchers suggest that international initiatives aimed at reducing emissions by encouraging forest preservation should explore the option of transferring the ownership and management of larger tracts of forests to local communities.

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Screening of *Plagiochasma simulensis* Kash. for Antifungal Activities from Western Ghats of Maharashtra

J.K. Kashid, M.B. Kanade, A.B. Telave, S.S. Deokule* and S.J. Chavan

Post-Graduate Research Centre, Department of Botany, Tuljaram Chaturchand College, Baramati, Dist. Pune-413 102 (M.S.)

*Department of Botany, Pune University, Pune-411007 (M.S.) E-mail : jayramkashidpatil@rediffmail.com

Present work is depends on antifungal activities of liverwort *Plagiochasma simulensis* Kash. The aqueous, acetone, n-butanol and methanol plant extracts examined for inhibitory activity against four fungal pathogens like *Aspergillus niger, Fusarium graminearum, F. moniliformae* and *Rhizopus stolonifer.*

Keywords : *Plagiochasma simulensis* Kash., Antifungal activity, Organic solvent fractions, Western Ghats, Maharashtra.

INTRODUCTION

Bryophytes remain unexplored longer time with respect to antimicrobial properties because of its rare and unique life cycle. Number of bryophytes are known to their medicinal (Chang and Shao, 1986), antibacterial (Gupta and Singh, 1971) and antibiotic (Chopra and Bhatla, 1990) activities. Recently, Asakawa (1994 and 2001) extracted sesquiterpenes, phenolics and terpinoids from bryophytes. In connection with this, in the present study we examined the effect of *Plagiochasma simulensis* Kash. extracts with aqueous, acetone, n-butanol and methanol against *Aspergillus niger, Fusarium graminearum, F. moniliformae* and *Rhizopus stolonifer* fungi to prove antifungal activity.

MATERIAL AND METHODS

The plant material of *Plagiochasma simulensis* Kash. was collected in the month of September, 2009 from Sinhagarh, region of Western Ghats, Maharashtra. The properly cleaned (10 g) of plant material was homogenized with 10 ml distilled water as well as organic solvents like acetone, n-butanol, methanol separately. Homogenized material was shake on rotary shaker (250-300 rpm) for 24 hrs and filtered it with double layered muslin cloth and make 20 ml final volume with respective solutions (pH - 7). Filtrate was stored in refrigerator for further use in antifungal assays.

Antifungal properties of extracts were tested against the aforesaid fungi by filter paper disc diffusion method (Ronald *et al.*, 1984). Sterile Whatmann No. 1 paper discs (6 mm) were saturated with respective extracts then dried and placed on fungi inoculated Sabourd's agar plates. On each agar plate three filter paper discs were kept. Antifungal activity was assessed by measuring the diameter of growth inhibition zone surrounding of the discs up to 72 hrs at an interval of 24 hrs at room temperature (28 to 30°C). The results were compared with Penicillin (50 µg/ml) treated as control.

RESULTS AND DISCUSSION

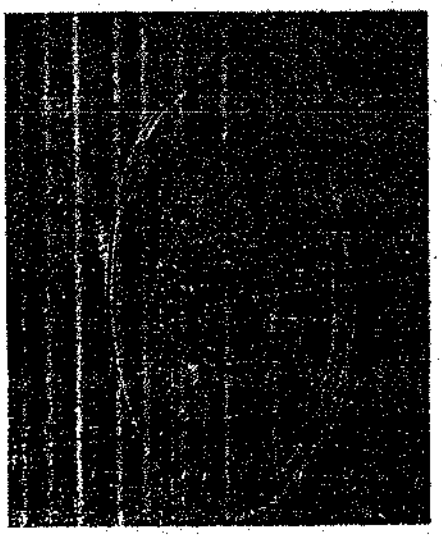
Results of the antifungal activity of *Plagiochasma simulensis* Kash. are depicted in Table-1 and Plate-1. The results were clearly indicates the growth inhibition of test fungi. In general acetone and n-butanol extract of *Plagiochasma simulensis* Kash. was more effective as compare to aqueous and methanol extracts. The maximum growth inhibition zone (13mm) was observed in *Fusarium graminearum* and *Aspergillus niger* of n-butanol and methanol extracts respectively. On the contrary, minimum growth inhibition zone was noted in *Aspergillus niger* (6 mm) of aqueous extract. It is interesting to note that, *Rhizopus stolonifer* growth was not responding to any extracts. Trace inhibition was recorded in *Fusarium moniliformae* of aqueous, n-butanol and methanol extracts and in *Fusarium graminearum* of acetone and methanol extracts. All results were compared with Penicillin (50 µg/ml) as a control but growth inhibition of all test fungi was altogether absent in it.

Joshi *et al.* (1990) observed the effect of *Exermotheca tuberifera* Kash. extracts on 04 bacterial strains *viz.*, *Escherichia coli, Klebsiella pneumonae, Bacillus substilis* and *Staphylococcus aureus* and reported growth inhibition of all test bacterial strains. The *in-vitro* antifungal activity of *Pallavicinea lyelli* was studied by Subhisha and Subramoniam (2005) against four test fungi (*Aspergillus niger, A. fumigatus, Fusarium oxysporum* and *Candida albicans*) using disc diffusion method. They concluded that water, alcohol and hexane extracts of *Pallavicinea lyelli* showed varying levels of

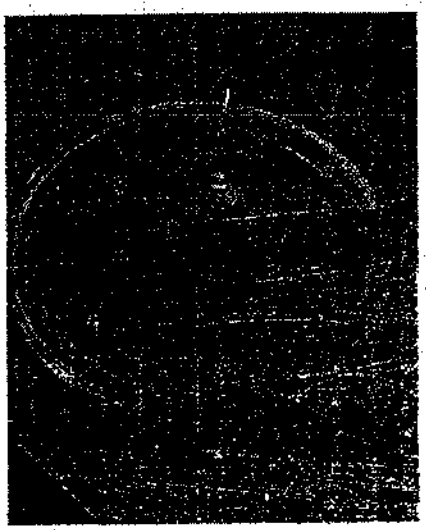
Antifungal screening of *Plagiochasma simulensis* Kash. against test fungi using disc diffusion method.

Plagiochasma simulensis Kash. extract	Diameter of inhibition zone of fungi (mm)			
	Rhizopus stolonifer	*Fusarium moniliformae*	*Fusarium graminearum*	*Aspergillus niger*
(Penicillin 50 µg/ml)	–	–	–	–
	–	±	–	6
	–	8	±	9
	–	±	13	9
	–	±	±	13

Plate 1 : Antifungal effect of *Plagiochasma simulensis* Kash. extracts against fungal strains.

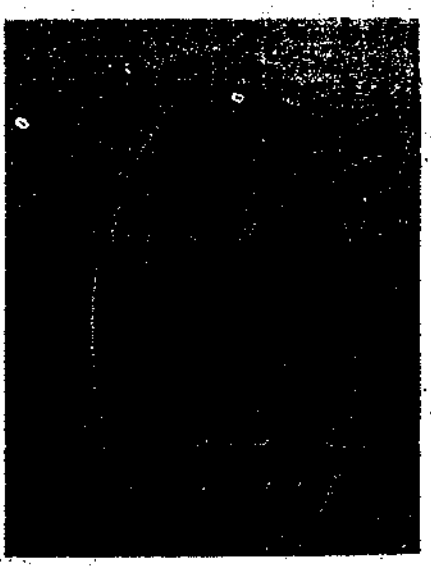


Fusarium moniliformae
(acetone extract)



Fusarium graminearum
(n-butanol extract)

Aspergillus niger
(n-butanol extract)



Aspergillus niger
(methanol extract)

ity against the test fungi; the alcohol extract exhibited the mum activity. Out of four fungi, *Aspergillus fumigatus* was d to be most sensitive.

Different liverworts and mosses screened to determine r antimicrobial activity against the selected bacteria and gal pathogenic species were studied by Bodade *et al.* (2008) recorded promising effect on growth inhibition of test anisms. Aqueous, methanol, n-propanol and petroleum er extracts of 40 Cyanobacterial isolates belonging of nine lera were examined for inhibitory activity against five fungal hogens (*viz.*, *Aspergillus niger, A. flavus, Colletotrichum isae, Paecilomyces lilacinus* and *Fusarium oxysporum*) by war and Puranik (2008).

The antimicrobial activity of bryophytes depends on the ysiological age of the species, season of the collection and ological conditions in which they are growing (Joshi *et al.*, 990). Therefore variations are observed in such studies. In e present work we also find out the antifungal activity of *egiochasma simulensis* Kash. against four test fungi. It is oped that this piece of work will unravel many intricate oblems pertaining to antibiotic properties of liverworts and ll also give a guideline to feature work.

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NUTRIENT CONTENTS IN THE RHIZOSPHERE OF THALLOIDS AT WESTERN GHATS, MAHARASHTRA

J. K. Kashid and S. J. Chavan

Post-Graduate Research Centre, Department of Botany, Tuljaram Chaturchand College, Baramati, Dist. Pune - 413-102.

ABSTRACT

The available nutrient concentrations varied at different places where liverworts grew.

Key words : Nutrient elements, Hepaticae, Rhizosphere soil, Western Ghats

Bryophytes play significant role in nutrient cycling of an ecosystem. They absorb and accumulate nutrient elements from litter and compete with higher plants for available nutrients (Oechel and Van Cleve, 1986). During present investigation nutrient analysis of soil under the influence of plant litter was studied from Western Ghats of Maharashtra State.

Thalloid liverworts *Plagiochasma simlensis* Kash., *Cyathodium tuberosum* Kash., *Targionia hypophylla* L., and *Plagiochasma articulatum* Kash. from Rajgad and *Fossombronia indica* St., *Sewardiella tuberifera* Kash., *Plagiochasma simlensis* Kash., *Targionia hypophylla* L., *Cyathodium tuberosum* Kash. *Plagiochasma articulatum* Kash. and *Riccia discolor* Kash. from Purandar hill forts of Western Ghats, along with their rhizosphere soils were collected during February 2008 to August 2009. These soil samples were used for analysis.

The nutrient elemental analysis was undertaken at Soil Testing Laboratory, Someshwarnager, Krushi Vidnyan Kendra and Post-Graduate Research Centre, Tuljaram Chaturchand College, Baramati (Dist. Pune). Available nitrogen (N) was estimated following Subbaiah and Asija (1965), phosphorus (P) following Olsen et al. (1954), organic carbon percentage (C%) following Walkey and Black (1934) and potassium (K) following Jackson (1958). The pH was measured using pH meter and electric conductivity by conductivity meter.

The results obtained indicates that, the available N (561.94 Kg/ha) and K (654.08 Kg/ha) contents in rhizosphere soil of *Targionia hypophylla* Kash. was high, while it was minimum with *Fossombronia indica* Kash. (< 3.5 Kg/ha). Rhizosphere of *Targionia hypophylla* (Mich) L. showed higher available P (4.21 Kg/ha), while available organic carbon content in the rhizosphere of *Plagiochasma simlensis* Kash. was high (3.37 %).

It was concluded that the soils of Western Ghats are rich in organic matter with sufficient amount of available C and N for favourable growth of bryophytes.

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