Thesis Submission For Ph. D. Degree

Studies On Biological Activities Of Some Mosses: Isolation And Characterization Of Some Metabolites.

Submitted To

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1. Introduction

Bryophytes conquer a main place in the plant kingdom. The term 'Bryophyta' a Greek word derived from 'bryon' meaning a moss and 'phyton' means a plant. They are primitive and simple land plants. They do not possesses well-developed vascular tissue system. Their adaptation to a terrestrial mode of life is incomplete as water is essential in one stage hence, they are called plant amphibians. They mostly fond in rainy season in humid areas. They becomes abundant within a short period so conquer large areas. They are mostly cosmopolitan in distribution.

Mosses are an extremely evolved group of bryophytes conquering a unique position between non-vascular and vascular plants. The dominant nature of mosses amongst the bryophytes is due to their better morphological and structural complexities. They are the creators of terrestrial habitats from the aquatic environment. Their life cycle is completed in two phases namely gametophytic and sporophytic. Gametophyte is dominant phase which produces male and female gametes. Water is necessary for the fusion of male and female gametes. Sporophyte depends on gametophyte. It has a typical capsule developed on the apical portion of a stalk called seta. The mature sporophyte liberates the spores that germinates under favorable conditions and produces protonema, it is a beginning of gametophytic generation. Mosses are luxuriously spread in the Indian subcontinent and it has great species variation. The mosses spread in the India have received vary less attention throughout the past due to non-availability of literature. Many researchers complete their continued studies and added to the flora of India mosses. Mosses of the Bombay Presidency by Blater (1929), Mosses of Nainital by Chopra (1960), Foreau (1961) published the moss flora of the Palni Hills. Mosses of Mahabaleshwar and Khandala by Dabhade (1974). Chopra (1975) detected nearly 2,000 moss species belonging 56 families of 329 genera. Gangulee (1969-80) published a books on "Mosses of Eastern India and adjacent regions" which comprised 990 species. He also published a hand book of Indian Mosses (1985)

Mosses belong to class Bryophyta, which earlier also includes hornworts and liverworts but now hornworts and liverworts have separate divisions. Mosses are an advanced class of bryophytes which includes about 17,000 species of 900 genera and 89 families of 4 orders under 3 subclasses distributed in the world (Richardson, 1981). Mosses shows rich diversity in various states of India. According to records about 34 species of hornworts, 816 species of liverworts and 2000 moss species are found in India. The previous effort carried on

morphology and anatomy of mosses by Hooker (1849), Birdwood (1886, 1887, 1897), Dabhade (1988, 1998), Dixon (1909), Blatter (1909, 1929) and Sedgwick (1910, 1911, 1913) gives a first, second and third list of mosses from western india.

The effort done by Kashyap (1914) in India is valuable in this concern. In Maharashtra the Bryo-floristic work about mosses are very fragmented. Sedgewick (1910) published a list of 71 mosses collected by him, Kirtikar and Woodrow from Mahabaleshwar, Kasara, Pachgani and Purandar in Western India. Dabhade (1974) investigated the four species of *Bryum* from Western India providing their taxonomical information, habit, habitat and range of distribution. Bryology of India by Udar (1976). Gangulee (1985) in his Handbook of Indian Mosses listed 100 species including Acrocarpous and Pleurocarpous mosses with colour photo plates. Dabhade (1998) reported 87 species of mosses from Khandala and Mahabaleshwar. Morphology and classification of mosses was given by Buck and Goffinet (2000). Nair *et al*.,(2005) gives information about Bryophytes of Wayanad in Western Ghats. Chaudhary *et al.,* (2006) published Bryophyte flora of Gujarat. Lal (2005) published Checklist of Indian mosses and documented 1623 taxa of mosses. Chaudhary *et al*., (2008) described more than 100 moss species from Konkan region of Maharashtra.

Hile (2011) reported 8 terricolous mosses and 17 species of mosses collected from different localities of Kasara Ghat of Maharashtra. .Daniels *et al*., (2013) published the Bryoflora of the southernmost Western Ghats of India. Sandhya *et al*., (2014) worked on Bryophytes of Andhra Pradesh. Recently Alam (2015) compiled flora of India mosses and noted the occurrence of 1578 species of mosses which belongs to 21 orders, under 66 families and 328 genera. Later no valid work has been made to provide a complete numbers of mosses in India. Soman (2016) gives information about some epiphytic mosses of Mumbai. Recently, Magdum *et al.,* (2017) published check list of 128 species of mosses, including 59 genera, of 26 families which belongs to 11 orders, from Western Ghats of Maharashtra. In recent years there is a notable progress in bryophytes floristic studies in different parts of the World. These studies provided valuable and important records on their distribution and ecology.

Previously mosses are studied for its taxonomical and anatomical characters but present work on mosses is further extended to Physico-chemical properties of rhizosphere and non-rhizosphere soil for soil elements analysis, pH and electric conductivity (EC) from different localities of Western Ghats of Maharashtra. The soil analysis is done for nitrogen (N)

phosphorus, (P) potassium (K) and organic carbon (C) and also for micro elements like Zinc, Copper, Iron and Manganese. Rhizosphere and non-rhizosphere soils are screened for isolation and identification of fungi. Antifungal and antibacterial activities are carried out by using four fungal and four bacterial strains respectively. Isolation and characterization of metabolites of *Bryum coronatum* have been studied.

The uneventual distribution of many bryophyte species, are still ignored which will lost forever. Thus to say that even the Western Ghats ecosystem is being destroyed at a rapid rate without adequate conservation.

Uses and Importance of Mosses

Mosses are commonly used for wound dressing and also for hygienic purposes all over the world for humans and animals. (Hotson, 19121). Different mosses have found their use in different ethno medical applications in many traditional cultures. High air and water holding ability of mosses has supported their application for thermal insulation.

Sphagnum mosses also have been and still are used as stuffing material for food transportation, benefits for their use due to high water absorption capacity as well as antimicrobial properties against food – borne bacteria (Hotson, 1921). Bryophytes are having a certain important medicinal value so used in pharmacological products (Banerjee, 1979; Asakawa, 1981). Mosses demonstrate antifungal, antibacterial, antiviral, insecticidal, anti-tumor, antioxidant, antiplatelet, anti-thrombin, neuroprotective activities and cytoxicity against cancer cells (Spjut *et al.,* 1992; Cheng *et al.,* 2012). One of vast scale application fields of mosses could be their use as alternative for peat, used as a growing media. Mosses used as substrate for growing orchids as it provides the necessary moisture levels and also some warmth that helps development of the plant. Asakawa (1994) reported phytochemicals from mosses which having antimicrobial, antifungal, anticancer and diuretic properties. Many compounds isolated from mosses have shown extraordinary biological activity, so extract of mosses are prospective for search of novel pharmaceutically active compounds.

The need is for studies associated to the many biological adaptations and processes. The mosses can be medicinal important, so some mosses are used as medicine to cure various diseases. The mosses can be ecologically importance, as they help in altering pH of soil, regulating nutrient cycling, creating soils, pioneer of vegetation and reducing soil erosion. They

are tolerant to pollution. This sensitivity of mosses makes them as a good indicators of air and water quality.

The Chinese, Native Americans and Indians have used number of mosses as herbal drugs for curing numerous skin disorders Ando (1983, 1984). The most widely known examples are the use of dried *Sphagnum* powder is sold to treat hemorrhages, *Rhodobryum gigantean* and *R. roseum* are used to cure nervous and cardiovascular diseases, *Polytrichum commune* to fever, as a diuretic, laxative, reduce inflammation and hemostatic agent (Asakawa *et al*., 2007). Transgenic *Physcomitrella* is now being used for production of blood clotting factor IX, for the cure of hemophilia B. Extracts of *Poytrichum juniperinum* had anticancer action against Sarcoma 37 in mice (Saxena and Harinder, 2004).

There is also evidence for mosses that their extracts help against varied types of illnesses such as burns, snake bites, neurasthenia, convulsions, pneumonia, tuberculosis, scald and others (Frahm, 2004, Singh *et al.,* 2006 Asakawa *et al.,* 2013) . Mosses can be found everywhere where human beings are living and it has influenced their use historically for different purposes. Mosses are also used for animal feed at polar circle (Glime, 2007). Moss lipid fraction has shown both antifungal and antibacterial activities against certain bacteria like *E. coli, C. albicans, M. smegmatis* etc. (Cansu *et al.,* 2013).

Mostly studies indicated that biological activity such as: cytotoxicity, anti–HIV, DNA polymerase β inhibitory activity, antimicrobial, antifungal, nematocidal activity as well as, insect antifeedant activity is due to the certain terpenoids and aromatic compounds found in mosses. *Polytrichum* moss species also used to stimulate hair growth, *Polytrichum* commune have diuretic activity, antipyretic and antitodal activity. Some mosses also used as sedatives and for different heart problems (Asakawa, 2007 Cansu *et al.,* 2013).There are same references of broad spectrum antibiotic activity in mosses. They have been also reported for their antibacterial activity against gram +ve and gram –ve bacteria.

The mosses produces a number of compounds like terpenoids, which shows remarkable biological activity, such as allergenic, cytotoxicity, insect antifeedant activity, insecticidal, neurotropic, microbial activity; anti-HIV, ant obesity and muscle relaxing. Mosses being rich source of a various secondary metabolites viz; alkaloids, tannins, glycosides, polyphenols etc. could be a hopeful source of the bioactive compounds with enormous therapeutic potential

Area of research

The present research provides the first hand consolidated account of 10 moss species belonging to nine genera, distributed over eight families. The sequential placement of the families and genera are according to Schuster's system of classification (1958c, 1979). This study is totally based on the material collected during frequently visits to the chosen localities of Maharashtra. The detailed work were done for identification, Physico-chemical and biological characteristics of mosses associated soils, antimicrobial screening of extracts and isolation and characterization of metabolites of selected moss.

The literature related to this work has been referred from Library of T. C. College Baramati. Dist. Pune, personal library of my research guide and websites. The map of Maharashtra and India showing the localities of collection have been adopted from Readers Digest 'Great World Atlas' and are based upon Survey of India Map.

Need of present study

The present work undertaken in a less explored area of the Maharashtra, among the hotspots of biodiversity. The lack of an easy to use identification manual is the key reason that made maximum of the students and teachers undertaking this subject. The present study may fill this gap to particular extent and can kindle more mosses studies. At present nearly 5% of the bryophytes have been identified and studied for their bioactive compounds.

The main challenges that modern world facing is a bacterial resistance to antibiotics. The number such type of bacteria as well as their resistance is increasing, more new substances that could be used to fight them are required. Very rarely lower plants like mosses are used for production of medicines and often only higher plants are considered to be of pharmaceutical value. This belief has left mosses ignored for many years, but in last 20 years many investigation have started to find chemical composition with their possible biological activities (Zinsmeister *et al.,* 1990., Harris, 2008;).

Due to rapid multiplication rates, high population sizes and the ability of these populations to respond to environmental changes enable bacteria to rapidly develop resistance to antibiotics. This led to emergence of drug resistant microbe such as methicillin resistant *Streptomyces aureus* and multidrug resistant strains of *Klebsiella pneumonia.* It creates serious challenge to the medical field to discover alternative and more effective medicines for the

treatment of such microbial infections. Such alternative antimicrobial molecules include bioactive compounds from natural sources like lower plants because of several reasons.

More commonly mosses extracts are tested for their antimicrobial property but there are also some proof of new types of biological activity. There has not been yet identified one specific substance that could be used as antibiotic, but the research continues and many moss extracts show this activity. In India recently some efforts has been done on antimicrobial activities of mosses (Alam *et al*., 2011, 2012) and interesting results have been observed, therefore more research on this aspects of mosses is needed.

Western Ghat is a rich biodiversity region of Maharashtra with enormous number of moss species spread in different habitats. Identification of these mosses is an urgent need. Furthermore these should be evaluated for their antimicrobial activity as several local reports are available from tribal people. The present work has been under taken to collect and identify the mosses of Western Ghat and also to screen these mosses for antimicrobial activity. The present work is further extended to find out the chemical profile of one dominant moss species which can be used further for therapeutic use.

The architecture / Frame

The overview of this research is presented here in the form of thesis. The thesis divided into 5 chapters. The first chapter is introduction which includes previous works related to the mosses study in different part of world, India and in Maharashtra. Uses of mosses in human welfare. The information clearly justifies the need of present work.

Chapter two deals with review of literature with reference to antibacterial and antifungal activities of mosses at global and India level, especially at different region of Maharashtra. Isolation of fungi from rhizosphere and non-rhizosphere soil, metabolites of mosses, applications, active compounds and their characterization in bryophytes specially noted in mosses.

The third chapter gives an information about materials and the methodology adopted during the study. It has been divided into various parts. These all parts includes different technique like study of habitats, collection and identification of mosses, physicochemical and biological characteristic of mosses associated soil, antimicrobial screening of mosses, isolation and characterization of metabolites.

Chapter four gives a detailed explanation of results and discussion. It is a major part of the work. The results of present research are provided in suitable manner and are discussed in.it. It gives detailed information about identification of specimens and verification through comparison with the preserved material stored at Laboratory. The selected 10 moss species are used for analysis of physicochemical and biological properties of mosses associated soils. In this chapter, the physical properties like soil colour, soil texture, pH and (EC) electric conductivity from each localities are documented. Elemental analysis of mosses associated soils are also carried out by using different methods. Fungi from rhizosphere and non-rhizosphere soils are isolated and identified, antibacterial and antifungal activities are carried out by using four bacterial and four fungal strains respectively. Isolation and characterization of metabolites of *Bryum coronatum* is carried out by taking certain phytochemical tests, HPLC, GC-MS, FT-IR and by using some parameters.

The last chapter is on summery and conclusion in which significance findings are briefly summarized. The literature sited in the present study is shown in bibliography at the end of the thesis.

Aims and Objectives of the research work:

The present work was undertaken to understand and enhance the knowledge about mosses from different localities such as Bhimashankar, Purandar, Sinhagad, Kaas; Satara, Lonawala, Khandala, Lawasa, Mahabaleshwar, Pachgani and Aundh of Western Ghats, Maharashtra, India.

Main aims and Objectives are as:

1) Collection, identification and selection of moss species.
2) To study physicochemical and biological properties of mosses associated soils.
3) To study the antimicrobial properties of selected moss species.
4) Isolation and characterization of metabolites of one moss species

2. Review of literature

2.1. About Mosses

Mosses are important group of ecosystems. There are near about 25'000 bryophyte species found in most of ecosystems worldwide, including mosses (Musci ~18'000 species), liverworts (Hepaticae ~6'000 species), and hornworts (Anthocerotae ~1'000 species). India is one of the extra-large biodiversity nations of the biosphere comes with an advantage of larger area and within its diverse bio geographical zones leading to great Indian floral diversity. Bio geographical zones of India are separated into seven regions with diverse climatic conditions, and topographical variations viz., (1) Eastern Himalayas, (2) Western Himalayas, (3) Gangetic Plains, (4) Punjab and West Rajasthan (5) Western and Eastern Ghats (6) Central India, and (7) Deccan Plateau and Andaman Islands. Recently, the eighth region, Andaman & Nicobar Islands in the Bay of Bengal is also added. Western Ghats are considered as to be one of the hotspots in the world.

Mosses are the simplest land plants, they belong to bryophytes. They are positioned in the second major taxonomic group of the plant kingdom. They are the major plant group in the plant kingdom, due to their abundance, adaptation strategies, unique biology and biochemistry. They can be found everywhere growing on various substrates and in a diverse of growth condition (Glime, 2007; Goffinet and Shaw, 2008). Recently Asakawa *et al*., (2013) shows interest in moss composition and their functions. Many compounds obtained from mosses have shown biological activity, so mosses extracts are potential for exploration of different pharmaceutically active compounds. Carbohydrates are main structural constituents of mosses, but they also contains secondary metabolites with great biological activity (Maksimova *et al*., 2013; Klavina, 2015).

Mosses are also highly resistant in respect to impact of UV radiation and pollution stress (e.g., impact of heavy metals) showing the existence of unique functions of their composition and secondary metabolites in their metabolism. Varied applications of mosses in pollution bio monitoring programs requires better understanding of processes governing pollutant accumulation in moss bodies and specially changes in their secondary metabolism.

2.2. Global status of antifungal and antibacterial activities of Mosses

Hayes (1947) was the pioneer worker who studied extracts of *Conocephalum conicum* (L.) Dumort. for antimicrobial activity, but, extracts do not showed any antimicrobial activity against verified pathogenic organisms. Madsen and Pates (1952) find out that occurrence of antimicrobial

substances in chlorophyllose plants growing in floridia. Later,on Pates and Madsen (1955) studied eight bryophytes viz. *Conocephalum conicum* (L.) Dumort, *Sphagnum strictum* Sull. *Dumortiera hirsuta* (Sw.) Nees, and *S portoricense* Hampe but found active against *Staphylococcus aureus* Rosenbach, *Candida albicans* Berkhout and *Pseudomonas aeruginosa* respectively. Extracts showed antibacterial activity against *Escheria coli* and some other microbial strains. Bryophytes like, *Dicranum scoparium* Hedw. (Moss), *Porella platyphylla* (L.) Pfeiff. and *Marchantia polymorpha* L were found considerably active against bacteria viz., *Bacillus subtalis* Cohn, *Sarcina lutea* Goodsir and *S. aureus* Rosen but *E. coli* do not showed any activity against tested plants.

Asakawa (1981), reported that numerous secondary metabolites are found in bryophytes e.g., herbertane–type sesqui-terpenoids and diterpenoids which exhibited antimicrobial activity against *Rhizoctonia solani* Kuhnand, *Phythium debaryanum* R. Hesse and *Botrytis cinerea* Pers. Methanolic extracts of *Odontoschisma denudatum* (Nees) Dumort and *Herberta adunca* (Dicks) Gray, containing antifungal substances like (+)–acetoxyodontos chismenol, (-)-α herbertenol, (-)-α formyl herbertenol and (-)- β herebertenol shows activity against pathogenic fungus like *Rhizoctonia solani*, Botrytis cinerea and *Pythium debaryanum* (Ando and Matsuo., 1982).

Castaldo *et al*., (1988) tested bryophytes like, *Conocephalum conicum, Mnium umdulatum* Hedw. and *Leptodictyum riparium* (Hedw.) Warnst, against eight pathogenic bacteria for their antibacterial potential and found effective.. Rodriguez *et al.,* (1996) studied antibacterial activity of mosses viz; *Sphagnum magellanicum, Hypnum amabile* and liverworts *Trichocolea tomentos* and *Metzgeria decipiens* against bacteria like *Bacillus subtalis, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* of commercial fruit juices and compared with Clindamycin and Ampicillin. Ethanolic extracts of studied plants showsed activity against minimum two of the assessed bacteria with varied degrees of inhibition. Basilea *et al*., (1999) isolated and identified seven pure flavonoids from studied mosses. The favonoids were the favones apigenin, lucenin-2, luteolin-7-O-neohesperidoside, saponarine, apigenin7-O-triglycoside, vitexin and the bifavonoid bartramiafavone. Out of these favonoids same shown antibacterial activities against *Enterobacter aerogenes, P, aeruginosa* and *E. cloaceae*. Zhu *et al*. (2006) described specific antibacterial activity against gram +ve and gram -ve bacteria from liverworts and in mosses.

Qu *et al.,* (2007) showed a major component riccardin C, of *Plagiochasma intermedium* Lindenb. & Gottsche which exhibited antifungal activity against *Fusarium oxysporum* and *Botrytis cinerea* in moderation at MIC value of 32µg/ml but three chlorinated bis (bibenzyls) viz. bazzanin S, isoplagiochin D and bazzanin B from *Bazzania trilobata* (L.) A. Gray, and isoplagiochin D, which was found most active one against fungi like *Phythophthora infestans, Cladosporium cucumerinum, Septoria tritici* and *Pyricularia oryzae*. Wu *et al*. (2008) isolated plagiochin E (PLE), from liverwort *Marchantia polymorpha* L and find out its result on cell wall chitin synthesis in *Candida albicans*. Cell wall was completely damaged, due to the antifungal activity of plagiochin E.

Veljic *et al.* (2009) tested antibacterial activity of *Fontinalis antipyretica* Hedw, *Hypnum cupressiforma* Hedw, *Ptilidium pulcherrimum* (Weber) Vain, and *Ctenidium molluscum* (Hedw.) Mitt and antifungal activity of methanolic extracts of *Ptilidium Pulcherrimum* against gram-ve and gram+ve bacteria and appreciable results were found compared to synthetic antibiotic and fungicide.

Veljic *et al*. (2010) evaluated antimicrobial activity of *Ptilidium pulcherrimum.* Antibacterial activity was evaluated invitro by using methanol extract against six fungal and five bacterial species. The extract revealed a stronger effect against tested Gram (+) than Gram (-) bacteria. Veljic *et al*. (2010) studied antimicrobial activity of three bryophyte, two mosses, and one liverwort. DMSO extracts was tested against bacterial strains *viz; Pseudomonas aeruginosa, Enterobacter cloacae, Salmonella typhimurium, Micrococcus flavus, Staphylococcus aureus Listeria monocytogenes, Bacillus cereus,* and *E coli.* All studied bryophytes extracts inhibits the growth of tested bacteria.

Elibol *et al*. (2011) studied six acrocarpous mosses for their antimicrobial effect, invitro against eight different microorganisms. Different solvents like methyl alcohol, ethyl alcohol acetone, and chloroform were used for preparation of extracts. Maximum antimicrobial effect was noted in methyl alcohol extracts and the minimum in chloroform extract. Standard antibiotic discs like Ampicillin (10 µg), Erythromycin (15 µg), Vancomycin (30 µg), and Ketoconazole (50 µg). were used to compare all results.

Filiz *et al*., (2011) assessed the anticancer property and antimicrobial activity of *Fontinalis antipyretica* Hedw. Extracts were prepared in methanol, chloroform acetone and ethyl acetate. Antimicrobial activity was assessed by using well diffusion method against eight bacterial and seven fungal strains. Extracts of acetone, chloroform and ethyl acetate shows activity against almost all the tested strains. They suggests the probability that *F. antipyretica* has anticancer and antimicrobial

agents. Altuner *et al.,(*2012) assessed in vitro antimicrobial screening of *Hypnum andoi* against test organisms and found significant results.

In Latvia, first time antimicrobial activity of eleven bryophytes species were evaluated by using ethanolic and aqueous extracts against *Bacillus cereus, Escheria coli and Staphylococcus aureus.* Both extracts showed remarkable activity against tested bacteria (Nikolejeva *et al.* 2012). Vidal *et al*. (2012) determined antibacterial activity of *Octoblepharum albidum* Hedw. against six bacterial strains by a micro dilution method. The extracts were prepared in ethanol only and in association with aminoglycosides. The results shown a similar inhibitory action against *E. coli and Klebsiella pneumonia* (MICs 512 µg/mL).

Bukvicki *et al*. (2012) find out antibacterial and antifungal activity of mosses like *Abietinella abietina, Cratoneuron filicinum Platyhypnidium riparoides, Neckera crispa* and *Campylium protensum* against *Penicillium ochrochloron, P. funiculosum, Trichoderma viride, Aspergillus flavus, A. fumigatus,* and *A niger.* The methanolic extracts of all studied mosses showed an antimicrobial effect against all tested microorganisms.

Savaroglu *et al*. (2011) tested antifungal and antibacterial activities of mosses like *Funaria hygrometrica*, *Polytrichum juniperinum, Tortella tortuosa*, *Hypnum cupressiforme* and *Hypnum imponens*. against three fungal and six bacterial strains. Agar diffusion assay and micro dilution methods used for testing activity. Extract of *Tortella tortuosa* indicated the maximum antibacterial activity against *Pseudomonas aeruginosa (5.9* MIC µg/ml). Mosses like *Tortella tortuosa*, and *P. juniperinum* indicated the greatest inhibitory activity against the tested bacterial and fungal strains.

Wakuli *et al.,* (2003) studied that the extracted pigments of bryophytes exhibited antibiotic properties against gram positive bacteria (*Aureobacterium liquefaciens, Arthrobacter globiformis, Bacillus brevis, B. cirulans, B. subtilis* and *Curlobacterium plantanum*). Catenarin also inhibited the growth of fungi accompanying P. tritici-repentis during the saprophtic phase of development. The most sensitive species was *Epicouim nigrum,* whose growth was inhibited up to 90 per cent.

Olofin *et al*. (2013) assessed antimicrobial efficiency of seven mosses and three liverworts. Extracts were prepared in ethanol, acetone and water. Bacterial strains like *Candida albicans, Staphylococcus aureus and fungal strains like Mucor rouxii* and *Penicillum notatum* were used to evaluate antimicrobial activity. Extract obtained from *Riccia flacida* inhibited the growth of *S.*

aureus, and *C. albicans. Cyatodium aficanum* showed activity against *Mucor rouxii*. The above results indicates that effective antimicrobial compounds can be sourced from *Riccia flacida* and *Cyathodium africanum*. Altuner *et al*.,(2014) screened antimicrobial activity of *Calliergonella cuspidate,Dicranum polysetum* and *Hypnum cupressiforme* against certain microorganisms and found significant results.

Yesiku and Caleb. (2015) studied antimicrobial activity of Nigerian mosses like *Racopilium africanum*, *Cyclodictyon sp,* and *Calymperes erosum.* on pathogenic microorganisms. Organic solvents like acetone, methanoland ethanol were used for preparation of extracts. Antimicrobial properties of above extracts were assessed by using agar diffusion method. Extracts shown antimicrobial activity against the tested microorganisms. Methanolic and acetonic extracts showed less activity than the ethanolic extract.

Canli *et al.* (2015) assessed the antimicrobial activity of *Mnium stellare* against one fungal and seventeen bacterial strains. Ethanol extract shows antibacterial activity against several Gram +ve and Gram -ve bacteria. Erturk *et al.* (2015) assessed the antioxidant and antimicrobial activity of eight moss species obtained from Turkey. The antimicrobial activity of extracts was verified against three fungi and six Gram (–) and four Gram (+) bacterial strains. All moss extracts showed activity against all the organisms, except *Homalothecium nitens*. Mosses like *E. striatulum* and *H. sericeum* showed the maximum antioxidant activity. Williams *et al.* (2016) studied antimicrobial activity of epiphytic mosses like *Pogonatum microstomum* Brid, and *Pallavicinia lyelli* (Hook), *Fissidens brevinervis (Broth.)* against Penicillin resistant bacteria. Various solvents like acetone, ethyl acetate and distilled water, were used for preparation of extraction. All extracts showed remarkable antibacterial activity against all tested bacterial strains.

Sevim *et al*., (2017) determined the antibacterial activity of twenty three bryophytes against *Paenibacillus* larvae which causes foulbrood diseases in larvae of honeybee. Methanol was used for preparation of extracts. Some mosses extracts showed remarkable activity. Junairia *et al.,* (2018) investigated the antifungal activity of mosses like *Hyophila javanica, Meteorium subpolytrichum, Dicranoloma reflexum, Dicranella coarctata Isotheciopsis comes, and Homaliodendron flabellatum* against *Candida albicans.* Extracts of all the mosses at different concentrations can inhibit the growth of *Candida albicans* except in the methanol extract of *Dicranoloma reflexum* and *Homaliodendron flabellatum.* All these results highlight mosses as probable source for production of

various herbal medicines. Hence, the global scenario of bryological research concerning antimicrobial properties is going fair enough, but in India this challenging aspect need special attention.

2.3. Indian status of antifungal and antibacterial activities of Mosses.

Mosses were ignored for their biochemical studies from a long time in India. Many chemicals present in mosses shows significant biological activity. Petroleum ether extracts of *Timiella and Barbula* were verified for their antibacterial activities against thirty three bacterial strains of Gram-positive and Gram-negative bacteria and significant positive results were found (Gupta and Singh, 1971). Dikshit *et al*. (1982) tested bryophytes like *Pogonatum aloides* (Hedw.), *Diplophyllum albicans* (L.) and *Plagiothecium denticulum* (Hedw.) Schimp for their antimicrobial activity and found remarkable activity.

In India, majority of the earlier bryologists were focused on the systematics of bryophytes, therefore, no significant work of past is known regarding antimicrobial activity. In latest times due to the global efforts few researches have been started to work on antimicrobial activities of mosses (Subhisha & Subramoniam, 2005; Bodade *et al.,* 2008; Kashid *et al.,* 2012; Deora & Guhil, 2014, 18).Mosses are the more studied one followed by thalloid and leafy liverworts. Banerjee (2000) carried out scrutiny of the antimicrobial activities of some bryophytes, which shows that nearly 200 bryophytic plants have been surveyed so far to identify such activity, of which 53-76% of the bryophytes shown positive results.

Bryophytes shown antibiosis activity against a numerous pathogenic fungi (Mekuria *et aI.* 2005). Subhisha and Subranomiam (2006) studied in vitro antifungal activity of *Pallavicinia lyellii* against four fungi viz, *Aspergillus niger, A. fumigatus, Candida albicans and Fusarium oxysporum* by using direct dilution and disc diffusion methods. Hexane, water, and alcohol were used for preparation of extracts. Extracts showed remarkable activity against the test fungi. Maximum antimicrobial activity was noted in alcoholic extract. Semra *et al.,(*2006) assessed the antimicrobial activity of *Palustriella commutata* extracts against test organisms and found significant results. Few members of mosses and hepatics have been scrutinized for their antibacterial activities and significant results were obtained (Kang *et aI,* 2007). Antimicrobial activity of some Indian mosses

was assessed by Rawat and Govindrajan (2007).They found significant results against test organisms.

Singh *et al*., (2007) assessed the antimicrobial activity of fifteen Indian mosses, against five Gram + ve and six Gram –ve bacterial strains. *Barbula arcuata, Barbula javanica, Mnium marginatum, Brachythecium rutabulum, Brachythecium populeum, Entodon rubicundus* and *Sphagnum junghuhnianum* and positive results were found against above tested microorganisms. Xie and Lou, (2008) stated that compounds obtained from bryophytes have shown contrary of conventional antibiotic resistance developed in human pathogenic fungi.

Mewari *et al*., (2008) screened antimicrobial activity *Marchantia polymorpha* L. against bacterial strains, like *Proteus mirabilis, Staphylococcus aureus* and *Escheria coli*, and fungal strains, like *Aspergillus flavus, Aspergillus niger, Trychophyton mentagrophyes* and *Candida albicans.* Crude extract was prepared in methanol. All above fungal and bacterial strains were found to be sensitive against the tested extract. Results indicates that the *M. polymorpha* can be used as antimicrobial drugs in the future.

Shirzadian *et al*., (2009) assessed the antifungal activities of two liverworts and twenty one mosses. Extracts were prepared in ethanol, methanol, petroleum ether, acetone and water. All extracts were mixed with Czapek-Dox medium at the ratio of 1:10. Fungal strains namely, *Alternaria alternate, Fusarium oxysporum, F. solani, Macrophomina phaseolina, Pythium sp* and *Verticillium dahliae* were grown on these prepared extracts. The ethanolic extracts of mosses namely, *Bryum pallens, Plagiomnium rugicum, Grimmia pulvinata, Philonotis marchica, Haplocladium sp.,* shown broad spectrum antifungal activity followed by liverworts like *Dumortiera hirsute* and *Pellia epiphylla.* Ethanolic extract shown the more activity than the other used solvents.

Deora *et al*., (2010) determined antifungal activity of a moss *Philonotis revoluta* against fungi viz; *Fusarium moniliformae* (Sheldon), *Curvularia lunata* (Wakker) Boedijn and *Helminthosporium turcicum* (Pass). Leonard & Suggs. They assessed fresh weight and colony diameter of test organisms under the effect of varied concentrations of extract. All the extracts showed remarkable inhibitory activity against tried fungi. Fresh weight and Colony diameter was found to be maximum in lower concentrations and minimum in higher concentrations of *Philonotis revoluta* extract. Sawant *et al*., (2010) assessed invitro antibacterial activity of the bryophytes by using agar-well diffusion method. Dichloromethane and methanol were used for preparation of

extracts. Three bacterial strains were used to assess activity. Inhibition of bacterial growth was compared with that of tetracycline and ampicillin as positive control and solvents used as negative control. Both the extracts of *Asterella wallichiana, Targionia hypophylla* and *Plagiochasma intermedium* shown antibacterial activity against all the tested bacterial strains.

Alcoholic (ethanol and methanol) and aqueous extracts of *Entodon nepalensis* Mizush and *Atrichum undulatum* (Hedw.) were assessed for its antibacterial activity against *Escherichia coli, Bacillus subtalis* and *Salmonella typhimurium* (Alam *et al*., 2012). Antifungal activity of extracts of *Hyophila rosea* Williams, *Plagiochasma rupestre* (J.R. Forst. & G. Forst.) Steph *and Targionia hypophylla* L. were tested against fungal strains like *Aspergillus niger, A. flavus, Alternaria alternata, Botrytis cinerea Phytophthora infestans, Trichoderma viride, Fusarium oxysporium, Penicillium expansum,* and *P. chrysogenum* and it was noted that these plants extracts were more potent antifungal agents than the synthetic fungicides at an average concentration of extract ranging from 2.5mg/ml to 20mg/ml (Alam, 2012).

Sharma *et al*., (2013) studied comparative antimicrobial activity of *Reboulia hemispherica* by agar well diffusion method. Activity was assessed by using Phenolic compounds and methanolic extract. The gram -ve bacteria were less sensitive than the gram +ve ones, while the fungal strains were less sensitive.

Deora and Guhil. (2014) investigated the effect of boiled and cold water crude extract of *Bryum capillare* against a *Drechslera maydis* which is a plant pathogenic fungus. Result showed that boiled water extract had strong antifungal activity against the test fungi. Relative study was also performed on bryophytes viz, *Anoectangium clarum* Mitt, *Hyophila spathulata* Jaeger and *Hydrogonium arcuatum* (Griff.) Wijk & Marg, for their antimicrobial activity against bacteria viz, *Bacillus subtilis, E. coli Staphylococcus aureus, and Enterobacter sp*. and fungi like *Trichoderma viride, Aspergillus niger and Fusarium solani* was found remarkably better activity as compared to synthetic drugs. (Alam, 2015).

Deora *et al*., (2016) assessed antifungal activity of a moss *Philonotis revolute* by hanging drop method. Methanol and acetone were used for preparation of extracts. The methanol extract of moss exhibited a greater effect against spore germination of fungus *Helminthosporium turcicum* than the acetonic extract.

Kandpal *et al*., (2016) tested the effectiveness of bryophytes as a substitute to the synthetic drugs by determining their biochemical and antimicrobial potential. Antibacterial and biochemical properties of a moss *Hydrogonium gracilantum* (Mitt), and two liverworts like *Reboulia hemisphaerica L.* and *Marchantia palmate* was assessed under laboratory conditions. Both ethanolic and acetonic extracts of the above moss and liverworts inhibited the growth of *Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli and Erwinia chrysanthemi* on an agar plate.

Deora and Suhalka. (2017) studied fungal toxicity of acetonic, methanolic and aqueous extract of *Riccia gangetica*. Extracts were prepared in acetone methanol and aqueous solution from 10-100 concentration. The fungal toxicity of extract was measured by spore germination percent, hyphal length and inhibition using hanging drop method. Maximum antifungal activity was noted in 100 per cent methanolic extract and other extracts revealed moderate to negligible antifungal activity. Pejin *et al.,(*2017) assessed antimicrobial activity of *Rhodobryum ontariense* against eight bacterial strains. Dimethyl sulfoxide extract of moss was tested by micro dilution method and found remarkable activity against tested bacteria.

Kadam Pratima., (2017) studied phytochemical screening and antibacterial activity of the bryophytes. The antimicrobial activity was evaluated against the two bacterial strains from the bryophytes viz *Asterella angusta, Plagiochasma articulata, Anthoceros erectus, Cyathodium tuberosum,* and *Targionia hyphophylla*. Highest antibacterial activity was showed by *Targionia hyphophylla*.

Deora and Guhil. (2018) studied the antifungal activity of *Bryum argenteum* against *Curvularia lunata,* which is a causal organism of leaf blight disease of maize. Pour plate method was used for assessment of extract. The percent inhibition and minimum inhibitory concentration (MIC) was determined. It was observed that the ethanolic extract have an extensive antifungal activity against the test fungi. A phytochemical screening of the extracts was also carried out to assess the antifungal substances.

2.4. Rhizosphere and non rhizosphere soil analysis for Microorganisms

This study will provides an idea about fungi associated with the rhizosphere and nonrhizosphere soils of mosses from Western Ghats, of Maharashtra, India. In this analysis, we will focus on the isolation and identification of both soil fungi. The rhizosphere is the region closer to

plant roots with associated soil matter and microorganisms. The rhizosphere zone can extend up to one cm or more from the root surface. It depends on the plant type, soil texture and moisture of soil. Relatively vary limited compilation of data are available on geofungi (Gilman, 1957., Ainsworth *et.al.,* 1973).

Roots associated with the rhizosphere soil secrete root-derived substances. These are classified as secretions (active process), root exudates (passive process), mucigel (root/microbial byproduct), and lysates. The addition of all these secretions into the soil is called rhizo deposition, which plays important role in process by which carbon is transmitted from living plants into the soil. Rhizodeposition increases the energy status of the rhizosphere soil and also the activity and population of soil microbes.

Relationships between bryophytes and soil fungi remains under-investigated. Besides antifeeding effect, bryophytes are also known to have different associations with microorganisms like protozoans, fungi, bacteria and algae. (Asakawa, 1990; Basile *et al.,* 1998; Sabovljevic *et al.,* 2001).The rhizosphere is a location that the plant himself selects where pathogenic or useful microorganisms create a major significant force on plants health and development. Microorganisms found in the rhizosphere includes protozoa, algae, fungi, bacteria, micro arthropods and nematodes. (Lynch 1990; Raaijmakers 2001).

Fungi are habitually associated with the rhizoids of bryophytes. A vast number of bryophytes have the benefits of fungal relationship, providing them with considerably more surface area for nutrients. Nearly 300 species of ascomycetes appear to grow as obligate on bryophytes. The bryophytes have never been found without fungal association (Doebbler, 1996).

Microorganisms shows variety of actions in the rhizosphere. Neutral or adjustable interactions include competition for nutrients, bacterial attachment, and free enzyme release. Harmful activities comprise phytotoxicity, allelopathy and pathogenesis. These positive, negative or neutral functions are ones that occur with associated roots and endophytic organisms. (Singh *et al.,* 2004). According to Read *et al*. (2000) bryophytes are well recognized to form association with fungi. Fungi are generally essential for the survival of other organisms at the individual as well as species and ecosystem level (Hawksworth 2001). Soil can be differentiated into two main types, in relation to plant growth namely rhizosphere and non-rhizosphere. Rhizosphere is the narrow area of

soil which touching the plant roots. The non-rhizosphere soil is a soil free from plant roots. It is the area where the soil make contact with plant roots, so considered as better microbial activities. It has been well-known that hornworts and liverworts form AM association. *Glomu sclaroideum* was studied for its harmful parasite grows inside liverworts *Marchantia foliacea* to form AM symbiosis was reported by Russell and Bulmon (2004).

There is remarkable association of bryophytes and fungi but very less devotion has been paid by bryologist. As per studies of Tapwal *et al.* (2004) the growth of bryophytes are influenced by mycoflora of non-rhizosphere soil but little attention was paid to bryophyte-fungal association. According to Hawksworth (2001) it is only 1.1% of the estimated total number of fungi. Only about 5% of the fungal species present in nature have been identified. About 16% of the 100000 species at present known are represented in culture collections (Crous, 2005).

Hyphomycetes and zygomycetes are mostly establish in the rhizosphere soil because they digest simple sugars. Microorganisms present in the rhizosphere soil control both physical and chemical status of soil profile that affect plants. The mucigel present in the rhizosphere plays significant role providing a bridge that prevents desiccation of plant by retaining the water column during water stress condition. (Sylvia *et al*., 2005).

The mycoflora nearby the rhizosphere differs from plant to plant and it is specific to the particular plant. Following are the most common fungal genera of rhizosphere soil such as *Aspergillus, Penicillium, Alternaria, Botrytis Cladosporium, Chaetomium, Cephalosporium, Fusarium, Gliocladium, Mucor, Rhizopus, Verticillium, Trichoderma, Monilia, Pythium,* etc. (www.agriinfo.in).The rhizosphere can also be referred as a combination of various soil particles and soil microorganisms, mostly bacteria and fungi. (Haghighi *et al.,* 2011).

Kashid *et al.,* (2010) screened rhizosphere soils of some liverworts for mycoflora from Rajgarh, Purandar, Sinhagarh and Rayreshwar of Western Ghats of Maharashtra. In total eight fungal genera were identified and their variations occurred as per altitude. The hill fort altitudes (basal, middle and high) seem to affect the density of mycoflora. Maximum fungal density of species was noticed at basal altitude and lesser at high altitude. *Penicillium notatum, Glomus fasciculatum* and *Aspergillus niger* were most common occurring fungi.

Maximum earlier studies on rhizosphere and non- rhizosphere soil bacteria and fungi have concentrated on crops plants such as amaranths, cassava, tobacco and okra. (Sule and Oyeyiola, 2012). Rhizosphere and nonrhizosphere fungi protect their host plants from pathogens, stimulate better growth and increases yield of crops. Rhizosphere is the biologically active zone of soil that surrounds the root and is biologically and chemically diverse from the surrounding bulk soil (Olahan *et al*.2015).There is remarkable association of bryophytes and fungi but very less attention has been paid by bryologist. The development in our ability to separate and to identify the soil fungi will help soil mycologists to know the facts of biological control, nutrient cycling and biotechnological demands.

2.5. Metabolites of Mosses.

Phytochemical means plant chemicals. It is a Greek word, "Phyto" means plant. Phytochemicals are classified as primary and secondary constituents, on the basis of their role in plant metabolism. Secondary metabolites are organic compounds that are not directly involved in the regular growth and development of a plants and other primary processes which are until not known. Secondary metabolites in a plant shows a key role in the existence of the plant in its surroundings and plays a key role in plant defense against other interspecies and herbivore defenses. Secondary metabolites are also used as medicines, recreational drugs and flavors. In addition, these compounds are also liable for the convenient effects on a selection of health related processes.

It has been confirmed that majority of the mosses contains mainly aromatic compounds like bibenzyls, benzoates, alkyl phenols, phthalides, naphthalenes, isocoumarins, lipophilic mono and diterpenoids and acetogenins. At present, more than 400 new compounds have been isolated and their structures studied (Asakawa, 1981; 1984; 1990; 1993; 1994). The medicinal properties of different plants are due to occurrence of several ingredients like alkaloids, flavonoids, terpenoids glycol-alkaloids, tannins, phenols, saponins, sesquiterpenes lactones, and esters. From above metabolites some act as synergistic and increases the bioactivity of other compounds. Due to universal occurrence, great diversity of species and unique chemical composition, bryophyte chemistry has not been commonly studied (Zinsmeister and Mues, 1990; Glime, 2007).

Antimicrobial potentiality and medicinal scenarios have not been revealed mostly till now, the study of literature point out that in bryophytes tannins, flavonoids, bioflavonoids, phenolic compounds, are active constituents which provides resistance against certain microorganisms and also acts as natural antioxidants (Hahn *et al*., 1995).

For analyse secondary metabolite, different extraction methods have to be used, because of varied compound polarities. As already mentioned, main interest in bryophyte chemical research is secondary metabolites with biological activity, extrahents mainly used for extract are methanol, ethanol, chloroform and water. (Basile *et al*., 1998; Sing *et al.,* 2007; Saboljevic *et al*., 2010; Fu *et al.,* 2012). It could be estimated that only 2% of known mosses and of 6% of liverworts have been analyzed for their chemical composition. Liverworts are studied mostly for their chemical composition because of their oil bodies which are not found in hornworts and mosses. Very few work regarding the bioactive components of bryophytes have been published (Basile *et al.,* 1999; Dulger *et al.,* 2005, Sabovljevic *et al*., 2006; 2008, Singh *et al.,* 2007; Veljic *et al.,* 2009; Mewari and Kumar, 2008; Elibol *et al.,* 2011). Secondary metabolites in defense may involves toxicity, anti-feedant activity and acting as precursors for physical defences. Secondary metabolites are widely used in plant protection, especially in Asia (Bodeker, 2000).

Mosses are a very interested group in botany but limited studies have successfully find out their chemistry, mostly at molecular level. Mosses generally grow in moist and shady places and their relative resistance to microbial attack shows their ability to produce some active antimicrobials (Asakawa, 1995; Bodade *et al*., 2008). Due to presence of secondary metabolites, various species of Bryophytes possess several biological activities, such as anti-fungal, anti-bacterial, anti-inflammatory and anti-oxidant properties (Saxena and Harinder, 2004).

Mosses are source for different secondary metabolites. In general, all these metabolites are a main source with a varied range of properties and structural arrangements. Secondary metabolic products according to Taiz and Zeiger (2006) containing a phenol group a hydroxyl functional group on an aromatic ring are designated as phenolic compounds. These form a chemically heterogeneous group of nearly 10,000 individual compounds. Deora *et al*.,(2008) assessed in vitro anti-fungal activity of *Plagiochasma appendiculatum* against *Alternaria solani* and found significant results. Mosses due to the occurrence of biologically active compounds in their composition are generally used in ethno pharmacology and for cure of burns and wounds (Singh *et*

al. 20011; Cheng *et al.,* 2012; Fu *et al*., 2012). Asakawa, (2007) mosses are rich in secondary metabolites like terpenoids, flavonoids, bibenzyles and fatty acid. Chemical composition of mosses varies according to species, season and growth environment. (Glime 2007; Goffinet & Shaw 2009; Xie *et al.,* 2009).

Some studies suggest that bryophyte phytochemistry can be used as a helpful tool to understand chemical composition of bryophytes, as well as to find and describe new proofs about the origin of lower and higher plants, with bryophytes being the link between them (Goffinet and Shaw, 2008; Glime, 2007; Asakawa *et al.,* 2013). One of the most dynamic feature that helped bryophytes to survive and retain their place in today's flora is due to their bioactive compounds (Bodade *et al.,* 2008).

Bryophytes, as a diverse group, is chemically still partially unknown although numerous new compounds were described only from liverworts. They have use in ethno-medicine, relatively rare comparing to higher plants and vary few uses are known in certain traditional medicine. The reports on biological activities of bryophytic extracts are neglected and unknown potentials of these second largest group of lower plants with 25,000 species and much more taxa worldwide (Sabovljevic., 2008). Generally, bryophytes are known to possess very high amounts of flavonoids glycosides, terpenoids, phenolic, fatty acids and certain rare aromatic compounds (Jockovic *et al*., 2008; Sabovljevic *et al.,* 2009).

Xie and Lou (2009) investigated the secondary metabolites in Bryophytes. Mosses contains a set of numerous unknown and known secondary metabolites. Secondary metabolites are the compounds present in particular cells that are not directly involved in metabolic processes like photosynthesis and respiration but are required for defense mechanism. They not only protect the plants from insect, pests, microbial attack but also function as drought tolerance, UV protection, and cold survival (Xie and Lou, 2009). Bryophytes have been confirmed to be a rich source of antibiotics, and efforts has been undertaken to find effectiveness, broad-spectrum, nontoxic antibiotics from these sources (Xie and Lou, 2009). Limited molecular level works find out some chemical compounds, like glycosides, terpenoids, phenolics, fatty acids and few aromatic compounds (Savaroglu *et al.,* 2011).

Adebiyi *et al.* (2012) reported that phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, steroids and phenols in variable quantities in the two moss plants but

phenol was absent in *Barbula indica*. These results recommend that the studied mosses can be genuine and potential source of useful drugs in various treatment.

Main part of moss phytochemistry is not only chemical composition analysis methods but also different extraction methods. By using various extraction methods diverse compounds can be detected, so extraction optimization is important part of studies. Now many methods can be used for extraction. Only limited studies have been explored for extraction conditions for secondary metabolites from bryophytes. But some extraction principles apply for both bryophytes and vascular plants some remain diverse, e.g. optimal concentrations of extrahent and treatment conditions (Klavina *et al*., 2015).

Wadavkar *et al*. (2017) observed that tannin and polyphenol were minimum in *Hypnum reflexum* while in *Steeriophyllum anceps* and *Brachymenium turgidum* those were higher. Klavina *et al.* (2018) find out that there was seasonal changes in composition of secondary metabolites and chemical composition in four moss species like *Sphagnum magellanicum, Sphagnum fallax, Pleurozium schreberi and Polytrichum juniperinum*. Dependency of both primary and secondary metabolic rate on climatic situations was confirmed. There are not many studies that take attention of biological activity of mosses and their possible reaction with other plant cultures due to their secretion of secondary metabolite in surrounding culture.

2.6. Application potential of mosses.

Mosses can be grow at all places where human beings are living and used historically for different purposes. More common and widely spread use of mosses is for wound dressing and sanitary purposes all over the world not only for animals but also for humans. In extreme situation mosses applications became sure, for example, as surgical dressing material in Japanese war, though more widely this type of dressing material was also used in World War I and II. That time huge amount of dressing material was prepared and improved by adding different chemical substances. Many mosses have found their use in different ethno medical applications in many traditional cultures Thieret (1956). Mosses are generally used as substrate for growing orchids as it provides the required moisture levels and also certain warmness that helps growth of the plant Perin (1962). In spite of high fiber content and low calorific value mosses are used as supplement in bread making or as soup preservative in native Indians culture, mostly in case of hunger has been reported (Bland,

1971). Large scale proportion of pharmaceuticals global are either genuine or modified naturally occurring compounds. Generally studies show that biological activity such as, antibacterial, antifungal activity, cytotoxicity, dNA polymerase, anti–HIV, insect antifeedant activity as well as nematocidal activity, is due to the terpenoids, phenols and aromatic compounds found in mosses. *Polytrichum commune* have antitodal and antipyretic activity. *Polytrichum* moss species also possesses diuretic activity and can be used to stimulate hair growth, some mosses can be used as sedatives and for different heart diseases (Zinsmeister *et al*., 1991 Asakawa, 2007; Cansu *et al.,* 2013). Singh *et al*.,(2000) published a review on medicinal importance of Bryophytes.

Bacterial species living in close association with certain bryophytes produce antifungal compounds and hence providing defense against microbial attack, so they can be used as biological tools in the controlling soil plant pathogens (Opelt & Berg, 2004; Xie & Lou, 2008; Beike *et al.,* 2010). Plant based natural commercial pesticides were developed from bryophytes due to their antimicrobial activities (Frahm, 2004). Bryophytes have been testified as antibiotic (Singh *et al.,* 2007; Shirzadian *et al*., 2009; Savaroglu *et al*., 2011a). Mosses have been used for cattle feeding though their calorific value is low and so commonly used as reindeer feed in areas like polar circle (Glime, 2007). Guo *et al*. (2008) the macro cyclic bis bibenzyls of bryophytes are generally used in controlling microbial infections.

Latest trends of people who prefer natural, organic, ecofriendly and chemical free food and other substances, moss usage could find its comeback. *Sphagnum* mosses have been still used as packing material for food transportation, due to their high water absorption capacity as well as antimicrobial properties against food borne pathogens (Mellegard et al., 2009). Due to high water and moisture holding capacity of mosses has kept their application as thermal insulation, therefore used in buildings in Latvian. As previously stated antimicrobial activity is the best tested parameter and it indicates great potential. Moss lipid fraction has shown both antifungal and antibacterial activities against *–E. coli, E. faecalis, S. aureus, B. cereus,* and *C. albicans* (Mellegard *et al*., 2009; Cansu *et al.,* 2012).

Beike *et al.* (2010) focus on some results that broadly open the doors for the use of diverse bryophytic species for plant biotechnology, suggesting that "Bryotechnology" is a rapidly evolving division of biotechnology in gene action of the bryophytes. Progress of biotechnology depends on difference of biological materials and identification of their novel application fields. From viewpoint

of new solutions in bio economy, mosses can be considered as earlier underestimated material but in future as a potential plant group in many fields starting as a new raw material and ending with prospective use in pharmacy. Several uses of bryophytes in bio economy are due to their basic properties like water and air holding capacity. Probable applications in bio pharmacy increase interest in bryophytic study from viewpoint of economic progress in the field of biomedicine and biotechnology. Aube *et al.,(*2015) find out that Sphagnum moss has potential use as a substitute for perlite and vermiculite in peat-based horticultural substrates.

Most likely one of large scale application of mosses could be their use as substitute for peat as a growing medium. This approach has been confirmed by many researchers and its efficiency has been proved by many studies (Kumar, 2017). Possibly it might seem as a new hint to use *Sphagnum* moss as a substrate, still many people are already using in their homes. Now situation has changed as far as appreciation of bio economy and its significance in future society. There is also proof for bryophytes that their extracts help against varied range of illnesses such as pneumonia, tuberculosis, neurasthenia, convulsions, scald, burns and snake bites also (Sabovljevic *et al*., 2016).

Ethnobryological uses are more curious because of their relative shortage, and for the perceptions they can provide the relation between people and small plants, like mosses, that invention on the limit of human awareness. *Polytrichum* and *Sphagnum* are the most frequently testified genera for their ethno botanical uses. This study realizes by conferring how these small plants can notify the study of ethno botany in general.

Historically mosses have been comparatively widely used, however, most of these application fields have not found their place in modern society as many other more effective solutions are existing. Now a days applications of mosses are so limited because of their small size, comparatively less effectiveness of their application in comparison to synthetic chemicals and constituents. Polyphenols and lipids are main chemical constituent groups that are assumed to have biological activity. More frequently mosses extracts are tested for their antimicrobial activity but it has been not yet identified one specific substance that could be used as antibiotic, but the research continues and many moss extracts shows this activity.

2.7. Research limitations of mosses

Mosses is the main taxonomical group of bryophytes. Due to their small size, it is difficult to collect sufficient amounts of plant material that is necessary for identification and chemical analysis of individual constituents. There are several mosses species which need microscopic check of a leaf cross section (often less than 1 mm in size). This makes collection of such species practically challenging for analysis of chemical composition (Zinsmeister and Mues, 1990; Goffinet and Shaw, 2008). Bryophytes play the vital role in understanding how the higher plants came to conquer land from water. Size of bryophytes is limited due to their anatomical characters. They do not produce substances necessary for building of strong cell walls such as cellulose based lignin and polysaccharides so they lack vascular system (popper and Fry, 2003; Goffinet and Shaw, 2008).

In most of the cases bryophytes have a tendency to form tight fitted colonies that slightly eases their gathering. At the same time these colonies often include few quantities of other higher plants or bryophytes, as well as small organisms, like bacteria, fungi, spiders, ants, and others (Glime, 2007). One of limitation in research of mosses is their proper identification, due to their small size and the unclear taxonomy (Glime, 2007; Goffinet and Shaw; 2008). Many compounds obtained from mosses have revealed great biological activity. Thus, extracts of mosses are potential for search of new pharmaceutically active compounds. Mosses might be considered as valued plants for development of bio economy. But, from huge number of mosses only few number of species has been widely studied (Asakawa, 2007).

Very little information regarding research about the chemistry of mosses is known and results is very scattered. The reasons for this are the difficulty in their proper identification and limited quantity of the same species available for analyses and the difficulty with which analysis can be accompanied since it relies on sophisticated methods. The interest in moss composition and roles in the environment is increasing day by day due to the occurrence of various biologically active compounds in their composition recently has been confirmed (Asakawa *et al.,* 2013).

3. Material and methods

3.1. Habitat analysis

3.1.1 Study area

Maharashtra is a third biggest state of India, both in area and population. It has western coastline extending 530 Km along the Arabian Sea from Goa. The Western Ghats are not true mountains but, are the edge of the Deccan Plateau. Its length is about 1,600 km, North -South and width 100 km, East-West. Climate in these Ghats fluctuates with distance from the equator and altitude. The climate is tropical and humid in the lower region and temperate closeness to the sea. Elevations is 2,000 meter and above in the south and 1,500 meter and above in the north. Average yearly temperature is around 15 °C. In some regions, frost is common and temperatures drop the freezing point during the winter season. Average temperature range from 24 °C in the North and 20 °C in the South region. Number of hill stations and forts are situated in Western Ghats of Maharashtra and these are selected for our study. The Western Ghats ranges from the Satpura in north border of Maharashtra to South past Goa, through Karnataka, Tamil Nadu and Kerala. The major hill ranges starting from North is the Sahyadri ranges. Maharashtra has a geographical place of 3, 07,713 sq. Km and is restricted with the support of East longitudes 72°30’ and 80°30’and North latitude 15° 40’ and 22°00’. Physiographically it may be separated into three natural divisions - the Western Ghat or the Sahyadri ranges, the coastal belt known as Konkan and the plateau. The Konkan consists rising and falling low lands. The Sahyadri slopes are lightly descending to the east and south-east.

Maharashtra has typical monsoon climate, with hot, cold weather and rainy seasons. Maharashtra receives its rainfall mostly from south-west monsoon. There is difference in rainfall. The monsoon season is in between June and September, winter in October and January, and summer in between February and May. Summer is extremely hot in months of April and May. Temperature varies between 22 °C to 42 °C during summer season.

Rainfall starts usually in the first or second week of June. July is the raining month, while September also gets sufficient rain. Monsoon starts its departure with the coming of

September to the state. Winter season is a cool, temperature varies between 12 °C and 34 °C with clear sky and pleasurable weather prevails from November to February. Rainfall in Maharashtra varies from region to region. Bhima, Krishna, Godavari and Tapi are the main rivers of the Maharashtra.

The soil of Maharashtra is formed from the underlying basalt rock. The soil is of black-cotton soil having high water retaining capacity. Soil set down along the river basins, the black cotton soils are heavier and deeper. The plateau areas have pathar soils, which contain greater gravel.

3. 2. Collection and Identification of mosses.

3.2.1. Samples Collection

The present work is based on the mosses collected from different localities such as Bhimashankar, Kaas-Satara, Khandala, Lawasa, Lonawala, Mahabaleshwar, Pachgani, Purandar, Aundh and Sinhagad regions of Western Ghats of Maharashtra State. Samples were collected from the corresponding sampling site (Figure 2.1.) in the July-September 2015–2018. Site with the homogeneous coverage of the corresponding moss were selected. The material was collected from shady places along the sides of foot paths, tree trunks wet walls and wet soils, during rainy season. Each moss sample (200 g) was collected in growing season. Moss samples were squeezed to get rid of excess water before collecting. The mosses were collected in polythene bags, covers with locality and field numbers. The field data were noted in the field note book such as date, locality, field number, habit, habitat any other significant characters associated with plants. Mosses were collected from following localities.

Aundh: This Village is in a Khatav Taluka of Satara District of Maharashtra, India. It comes under a Paschim Maharashtra region. It is 45 KM away from Satara city. It is 9 KM away from Khatav and 264 KM from Mumbai. Altitude is 623 meters above sea level.

Bhimashankar: Its location is 50 km north of Khed Taluka, near Pune district of Maharashtra. It is situated in the Ghat region of the Sahyadris ranges, 127 km away from Pune. The river Bhīma originates from it. It is one of the important wildlife sanctuary of the Western Ghats. Its area is of 130.78 km. It is rich in variety of flora and fauna. A variety of plants, insects, birds and animals

can be seen. It is located at 19°132"N, 73°554"E and about 15-16 km west of Nasik in the Sahyadri ranges, it is approximately 4250 feet above the sea level.

Kaas; Satara: It is about 25 km away from Satara. It is a plateau located at high hill and grasslands changes into a 'valley of flowers' in monsoon season. It has more than 150 types of flowers, grasses and shrubs. The orchids flowers here for 3–4 weeks during this period. This plateau is a World Natural Heritage place which is a part of the Sahyadri. The plateau shows a natural cycle of extreme conditions, with damp water-logged cool monsoons, very dry warm summer (45 °C) and cold winter (5 °C). The soil is acidic with thin layer on top of laterite rock beneath. Region has geographically located at 17°42'0"N, 73°50'E.

Khandala: It is well-known town lies only 5 Km away from Lonawala. It is relatively small than Khandala and blessed with natural beauty and plenty. High hills on one side and deep valley on the other side divide Khandala and Lonawala. It is located at 17° 42' 0" N, 73° 50’ 0" E.

Lawasa: Lawasa is situated near Pune, Maharashtra, behind the Varasgaon Dam of Western Ghats. It is a private and planned city. It is about 65 Kms away from Pune and about 200 Kms from Mumbai. Temperature ranges from a minimum of 6 º C in winter and maximum of 36 º C. in summer. Lawasa City covers 25,000 acres with 60 Kms of lakefront. It located 18° 24′ 19.01″ N, 73° 30′ 22.57″ E.

Lonawala: It is located on the Mumbai-Pune highway. It is only 5 km, away from the western hills of Sahyadris, Temperatures ranges from 12 °C in winter to around 36 °C in summer. The annual rainfall averages 450 cms. The soil is acidic, red laterite with thin layer on top. Lonawala lie at 18°44’59"N, 73°25'2"E.

Mahabaleshwar: It is a huge plateau of western Ghats measuring about 150 km and bounded by valleys on all edges. It is located around 285 km from Bombay and 120 km southwest of Pune. Height of ‘Wilson Point’ is 1,438 m at its maximum peak above sea level. It has an average height of 1,353 meters (4,439 ft). It is situated at 17°55'N 73°40'E, 17.92°N 73.67°E.

Pachgani: Pachgani is a well-known hill station in Satara district, Maharashtra. The five hills adjoining Pachgani are crowned by a volcanic tableland, which is the second highest in Asia. These raised lands are known as "table land", which is a main part of the Deccan Plateau. It is placed at 17.925°N 73.8°E stands 1,293 m (4,242 ft.) above the sea.

Purandar: It is 20 km away from Purandar (saswad) and 50 km away from Pune, Maharashtra. The relative humidity is maximum during mansoon which favours growth of bryophytes in such area. The fort of Purandar stands at 1,387 m (4,472 Ft) above from the sea level in the Western Ghats. It is situated at 18°16'50"N, 73°58'27"E 1,387 m.

Sinhagad: This fort is placed nearly 30 kilometers southwest of the city Pune. Formerly called 'Kondana' was also purposefully sited at the center of a sequence of other forts like Rajgad, Purandar and Torna. This fort is situated at 18°21'56"N 73°45'20"E, in Sahyadri ranges on a isolated cliff of Bhuleswar range at a height of 1350 m above from the sea level.

3.2.2. Pre-treatment of samples

After collection, the mosses were brought to laboratory as early as possible. They are separated from soil, stones, protozoans and other mosses. Selected mosses were cut off and put into plastic bag. Before analysis, the materials were dried at ± 40 °C, at room temperature and then were also preserved in paper packets. The preserved specimens along with necessary details were maintained in the Department of Botany, Tuljaram Chaturchand College, Baramati, Dist. Pune- 413102, Maharashtra, India.

3.2.3. Identification of Mosses:

Moss samples were brought to laboratory for analysis as soon as possible (usually within the same day). After proper cleaning and drying of mosses, investigation from various angles are carried out, which resulted in proper identification. Fresh mosses materials were used for identification. Morphological structures were studied under a dissection microscope. Size of leaves, apex, margin and capsules were measured. It was completed by comparing with the type specimen, referring authentic literatures (Dabhade 1998) and also by consultation with experts. For identification purposes sampled moss specimens were put into paper bags, labelled and put for a storage in Department of Botany, Tuljaram Chaturchand College, Baramati, Dist. Pune-413102, Maharashtra, India.

The mosses were also identified by western circle botanical survey of India, Pune. In this study mosses are separated by their growth habitats- *Fissidens crenulatus* Mitt, *Funaria hygrometrica* Hedw, *Hyophila involute* (Hook.) Jaeg, *Macromitrium sulcatum* (Hook.) Brid, *Bryum ghatens* Broth. ex Dix, *Bryum coronatum* Schwaegr, *Brachymenium turgidum* Broth. ex

Dix, *Trachypodiopsis blanda* (Mitt.) Fleisch, *Stereophyllum ancens* (Bosch. et Lac.) Broth, *Hypnum reflexum* F. E. Tripp. (List of moss species and growth conditions are in Table 2.1.). Rhizosphere and non rhizosphere soil Samples were also collected in plastic bags.

3.2.4. Descriptive key for identification of mosses:

A comprehensive key to the orders and families of mosses is given and also keys have been provided for all the genera in each family for easy identification. A key is purely based on reliable characters. The genera arranged in sequential manner. (Dabhade, 1974)

Key to the orders

A. Acrocarpous mosses. Peristome, when present, single (Haplolepideae)

Leaves mostly elongate to subulate; cells usually rectangular to linear, smooth.

Peristome teeth 162-pronged; dorsal layer thin, mostly clearly longistriate -------Dicranales

Leaves distichous, each with a dorsal wing, other characters as above ----------Fissidentales

Leaves mostly elongated and broad; cells less or more preponderantly

isodiametric and papillose. Peristome teeth 2-pronged, mostly with

thickened papillose dorsal layer, exceptionally double, sometimes absent ----------Pottiales

Leaves mostly lanceolate, often hair-pointed; cells small mostly

parenchymatous, often with undulate walls Peristome without a basal

membrane, teeth generally not 2-pronged ----------Grimmiales

B. Growth form predominantly acrocarpous. Peristome, when present

usually double (Diplolepideae) Process of the endostome on same radii

as the teeth of the exostome ---------- Funariales

Process of the endostome alternate with the exostome teeth ----------Eubryales

C. Growth form predominantly pleurocarpous. Peristome, when

present, double. Process alternate with the teeth. Either growth

habit acrocarpous, with cells in the middle of the leaf short, in

the Peristome teeth, the outer layer thicker and inner thinner or

growth habit pleurocarpous. ----------Isobryales

Stem often with distichous leaves, or flattened; Leaves mostly

asymmetrical, often bordered, binerved, Cells usually parenchymatous;

Capsule erect to inclined; Peristome double, rarely single or absent;

Calyptra often Fimbriate----------Hookeriales

Stem radial, seldom leafy. Leaves symmetrical or nearly so, not

bordered, single nerved or enervate; cells smooth or papillose,

parenchymatous linear in many cases; alae absent or defined.

Capsule erect, inclined or cernuous or pendulous; Peristome

double, inner often well developed; calyptra smooth ----------Hypnobryales

Capsule generally circular or angular as seen in T. s; Peristome

teeth apparently of horseshoe-shaped fibroid cells and united

at the top with the epiphragm---------- ----------Polytrichales

Key to the Family

Plants either small or larger and simple or dimorphic and

less branched. Leaves distichous and flattened in one plane, split

to nerve on the inner side of the lower half into two blades clasping

the stem, Cells parenchymatous or prosenchymatus ----------Fissidentaceae

Plants small to larger, mostly in tufts. Stem usually with a

simple to forked or fasciculately branched. Leaves of variable shape;

nerve usually thick-walled, mostly papillose on both surfaces, from

mid-leaf upwards. Capsule cleistocarpous or usually peristomate;

Calyptra mostly cucullate. Spores usually small ----------Pottiaceae

Low annual and biennial earth mosses. Stem usually with loose

ground tissue and outer small, narrow, thick walled cells, mostly

simple,radiculose at the base. Upper leaves mostly large and rosette

-forming, soft to somewhat crumped, broad and concave; capsule

Mostly pyriform, erect or inclined to pendulous, many stomata on

the neck; Seta usually elongated. Operculum usually flat or

Slightly convex. Calyptra often cuculate ----------Funariaceae

Perennial, growing on rocks or on bark, dry to swampy ground or

decaying wood. Mostly tufted, sometimes Gregarious. Stem in T.s

mostly circular some times Pentangular leaves mostly in many rows,

lower usually small and upper large. Seta more or less elongate,

smooth. Capsule inclined to cernuous, sometimes erect, long or short.

Peristome teeth mostly dagger shaped, always hygroscopic; Operculum

convex to shortly conical. Calyptra cuculate, Small and falling off

early. Spores small and spherical. ----------Bryaceae

Plants slender to robust, greenish, yellow or blackish in color. Stem

in T.s circular to oval, with or without pore. Main stem Creeping,

with blackish rhizoids and more or less scale like lower leaves;

secondary stem mostly profuse, erect or decumbent, seldom pendent.

Capsule exerted, symmetrical, ovoid to sub spherical; Annulus

usually not differentiated. Peristome Double, operculum conical,

obliquely rostrate. Calyptera cuculate and naked or mitriform and

hairy. Spores mostly subglobose, small to large ----------Trachypodaceae

Stem and branches alike or little differentiated, sometimes

Asymmetrical, Capsule inclined to horizontal or pendulous;

Peristome mostly imperfectly developed; teeth transversely

striate; basal membrane usually high; cilia usually

well-develop----------Hypnoideae

Leaves dimorphic, differentiated into upper and lateral, lower

usually absent. Lateral always symmetrical; nerve mostly single,

never complete, some times furcate, double or nearly absent;

cells oval, seta short. Operculum apiculate to nearly rostrate.

Cilia mostly absent----------Stereophylloideae

Stem long, creeping; cells in the leaf base mostly elongated.

Capsule ovoid to oblong or cylindrical, rarely striped. Calyptra

Campanulate-mitriform, rarely cucullate. ----------Macromitrioideae

Key to the genera .The genera are arranged in sequential manner.

1) Neck not separated from the urn; the later circular in T.s- ----------*Pogonatum*

2) Peristome teeth divided into crura half-way or more;

Crura longitudinally striate or with spiral thickenings ----------*Fissidens*

Stem firm with a central strand. Leaves always less or

more nerved. Capsule with stomata. ----------*Fissidens* Mitt

3) Leaf cells mostly transparent or translucent; Peristome

Teeth divided and contorted. ----------*Hydrogonium*

4) Cells of the lamina coarsely faintly or finely papillose,

Never mamillose and extended; nerve strong ----------*Barbula*

5) Calyptra becoming obliquely placed and generally not

Lobed, Peristome generally double----------*Funaria*

6) Leaves ligulate or spatulate; perichaetial bracts

Slightly or not sheathing----------*Hyophila*

7) Leaves all alike, Capsule exserted. Basal cells

thick walled----------*Macromitrium*

8) Processes and cilia usually normal and well developed----------*Bryum*

Processes mostly rudimentary; cilia rudimentary to absent----------*Brachymenium*

9) Leaves mostly auriculate. Seta up to 18cm long; Basal

membrane low. Leaf cells extend to linear margin,

Faintly to strongly serrate----------*Trachypodiopsis*

10) Lateral leaves mostly asymmetrical; nerve strong,

reaching to below the apex----------*Stereophyllum*

11) Basal cells of leaf usually thick walled and pitted;

Alar cells all alike----------*Hypnum*

Following mosses were identified and selected for study purpose:

1. *Fissidens crenulatus Mitt.*
2. *Funaria hygrometrica* Hedw
3. *Hyophila involute* (Hook.) Jaeg.
4. *Macromitrium sulcatum* (Hook.) Brid.
5. *Bryum ghatens* Broth. ex Dix.

6. *Bryum coronatum* Schwaegr
7. *Brachymenium turgidum* Broth. ex Dix.
8. *Trachypodiopsis blanda* (Mitt.) Fleisch.
9. *Stereophyllum ancens* (Bosch. et Lac.) Broth.
10. *Hypnum reflexum* F. E. Tripp.

3.2.5. Taxonomical characters

1. *Fissidens crenulatus* Mitt.

Plants small with reddish brown, stem up to 6 mm high. Leaves oblong –lanceolate, 1.5 mm long and 4 mm wide. Leaf broadest at base and acute towards the apex. Nerve light brown. Leaf cells with single, mamillose papilla, leaf margin more or less crenulate. Leaf base margin bordered by 1-3 rows of elongated, cells. Leaf base rounded at the point of attachment. Seta erect, apical, orange brown, about 5 mm long. Spores light brown, smooth and circular.

2. *Funaria hygrometrica* Hedw.

Plants lightly or closely tufted, 1-1.5 cm in high. Leaves yellow green, lower leaves smaller than upper, concave, lanceolate or wider obliquely placed, shortly pointed at apex, Laminar cells elongate, hexagonal to polygonal, long base with apical shorter region , little narrower at the margin. Basal cells larger, rectangular to sub-rectangular, polygonal and long, Seta long, reddish, and hygroscopic, strangely twisted after drying, brownish with yellow margin, orange or deep red, with a side mouth. Capsule symmetrical, inclined, oblique, pyriform gibbous at backstage, furrowed, wide mouthed when dehiscing, sulcate when mature, 3 mm long, 1.5 -2 mm broad. Peristome 5 mm long, complete, oblique and broad at base. Operculum convex. Spores brown and rounded. Capsule mouth asymmetrical red-brown wide, orange to yellow mouthed capsule is very easily recognized from other species.

3. *Hyophila involuta* (Hook) Jaeg.

Plants dark green with tuft of rhizoids at base. Stem height 1 – 1.5 cm. leaves long, lamina falcate, Leaf margin serrate or entire and mostly twisted when dry. Leaf cells small, quadrate. Seta erects, elongate, 2 – 2.5 cm high. Capsule erect, cylindrical or needle like, 4-5 mm long, lid conical. Spores rounded and light brown. Plants commonly grows on large stones of basalt on compound walls.

4. *Macromitrium sulcatum* Brid.

Marked characters of species are epiphytic moss growing on branches of trees with sulcate capsule and leaves with papillose cells. Red-brown plants with golden – yellow tip densely tufted, robust, glossy and bright. Branches 5 cm long. All leaves are similar, crowded, strongly contorted, erect, crispate when dry erect, 4-5 mm long, acuminate, transversely placed, acuminate. Leaf margin irregular, Leaf cells smooth, walls thin, rounded, incrassate, elongate in point, leaf basal cells narrowly rectangular, broad, pale yellow, strongly thickened. A small group of smooth cells present at the base of leaf by the side of nerve cells well differentiated from adjacent cells. Leaves sharply acuminate with more uniformly elongate areolation. Seta red, smooth walled, 10-12 mm long erect, soon becoming lateral. Capsule ovoid, small-mouthed, strongly sulcate towards base, exerted, Peristome double, calyptras naked, deeply laminate. Spores dark brown, light, globose to sub-globose.

5. *Bryum ghatens* Broth. Ex. Dix.

Plants tufted rigid, robust, deep-purple brown in color. Stem erect, thick, 7 mm -1 cm tall with sub floral innovations, Leaves stiff, erect to erect opatent hardly changed when dry, oblong-lanceolate, decurrent, acuminate, concave, with irregularly deflexed. Seta apical, erect, but arculate at tip. Capsule pendulant, deep red and excurrent. Seta, apical, erect. Moss can be identified by having tufted rigid, rebut, deep red or purple brown colour plants.

6. *Bryum coronatum* Schwaegr.

Plants grows on rocks. Dioeciously, tuft of slender dull, yellowish-green Stem 1 cm in height, with floral novelties. Leaves twisted when dry, ovate, concave and lanceolate. Capsule cylindrical, pear shaped pendulous, 3 mm high with distinct neck, Operculum slightly pointed,

teeth papillose, transversely striped, yellowish orange, spores slightly brown, oval or globose smooth margin. It is a cosmopolitan.

7. *Brachymenium turgidum* Broth.

Plant forming cushions with innovations at apex, growing on branches of trees. Plants erect, 1-1.5 cm high, resolute and caespitose. Leaves narrow, sub erect, twisted when dry, widely spreading and slightly falcate, ovate-lanceolate or ligulate. Leaf margin extremely narrow border. Leaf cell pellucid, elongated hexagonal and basal cells elongated to sub-rectangular. Nerve excurrent. Apices differ with allied species by the turgid, pendulous capsules.

8. *Trachypodiopsis blanda* (Mitt.) Fleisch.

Plants are epiphytic on branches of trees. Plants slender, greenish brown, densely tufted. Primary stem creeping, secondary stem pendulous, pinnate or bipinnately branched. Leaves closely placed, when dry spreading, erect. Apex acuminate with serrate margin. Sporophyte with oval capsule, seta 2 mm long, spores spherical to oval.

9. *Stereophyllum ancens* (Bosch et. Lac.) Broth.

Plant medium, green, main stem creeping, erect, 1 or 2 branched. Leaves laxly imbricate, elliptic-lanceolate, concave, acute apex and margin dentate at tip. Costa single, leaf cells rhomboid. Sporophyte formed on main stem, capsule erect or lightly bending, ovate, cylindrical. Long operculum conic short roseate. Spores small, yellow and smooth.

10. *Hypnum reflexum* F. E. Tripp.

Plants dull green, prostrate, creeping on bark of tree. Plant stems 2- 2.5 cm long, greenish brown, erect, irregularly branched, secondary branches narrow, procumbent, flatly leafy. Leaf cells rhomboidal, linear, apical cells papillose, cells ending projecting. Operculum projecting or short prostrates peristome double, exostome teeth straight and cross band at the end. Spores brown.

3.3 Physicochemical characteristics of mosses associated soils:

Physicochemical parameters includes Soil colour, texture, Electrical conductivity (EC) and pH.

3.3.1. Soil sampling and preparation of sample for analysis:

Soil samples separated from each mosses species brought to laboratory and spread out on an aluminum tray brown paper. Coarse, stones, gravels, rhizoids and other plant debris are removed. Big pieces 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 mesh. These samples were then used for study of various physicochemical characters.

3.3.2. Physical characteristics:

Physical characteristics like soil colour, texture, electrical conductivity and PH are measured according to standard procedure as given below:

A) Soil colour:

Colour of the soil is one of the key morphological feature. Color of the soil was recorded on the spot. The variation in colour of different horizons helps as one of the factors that hints to recognition of different soil types. Sometimes, colour of soil is partly-colored or multicolored. Moist soil is generally darker than when it is dry. Soil colour differs considerably. Some important soil colours are black, yellow brown, red, white and gray. Soil colour is due to colour of the predominant soil particles of weathering rocks and both organic and inorganic contents that impart colour to the soil mass as like below.

Black colour due to the addition of humus or decaying organic matter. Also magnetite and oxides of manganese and titanium, if present in sufficient quantities, also impart black colour. Red colour is mainly due to presence of iron oxide, in form of ferric oxide. Red soil 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 different hydrated iron oxides. Reddish brown soil is due to hydration of iron oxide and yellow brown, by hydrated goethite iron oxides. Gray colour soil is due to removal of bases like iron from the soil mass by leaching. It is formed in humid regions. White colour of soil is due to high contents of silica indicates a sandy soil.

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

It is usually done by crushing the soil lightly in a mortar and then material is passed through a 2 mm sieve to separate stones, gravel and small particles like silt and clay. The various soils can be grouped into three main factors viz. silt, sand 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 significant 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.

C) pH of soil sample:

Soil sample were dried at 60 ^{0}C for 72 h, crushed in mortar and pestle and filtered through 2 mm sieve and then sieved soils were dissolved in distilled water, filtered and then pH was measured by digital pH meter.

d) Electrical Conductivity (EC): It was measured by Electric Conductometer.

3.3.3. Chemical characteristics:

The elemental analysis was made at Soil Testing Laboratory Daund Sugar Pvt Ltd Alegaon, Daund Dist. Pune. Available nitrogen (N) is estimated by Kjeldahl method (2016); Phosphorus (P) by Olsen *et al*, (1954), Potassium (K) by Toth *et.al*. (1948) method and Organic carbon percentage (C %) by Walkley-Black (1934).

A. Macronutrients:

1. Nitrogen

For estimation of available nitrogen in the soil, Kjeldahl method (2016) was used. 50 g of dried powdered soil sample was taken in 500 ml Kjeldahl flask and then 1 g $CuSO_4$, 10 g K_2SO_4 and 30 ml Conc. H_2SO_4 was added in it. It was shaken until complete mixing and allowing to stand for at least 30 minutes with continuous shaking or until complete solution results. Content was digested up to the greenish colour appears. Digestion was stimulated on the Kjeldahl digestion rack with low flame initially 10 – 30 min until the bubbling stops and then steadily more strongly up to the sample is totally charred. Content allow to cool and dilute it with 100 ml distilled water. Fluid part is then poured to a 1000 ml distillation flask. Residue left in the Kjeldahl flask was washed with 4 or 5 times by 50 – 60 ml distilled water, transferring the washings into the distillation flask. Flask is fitted with two neck joints to one neck dropping funnel is connected for adding 40 % NaOH while to the other neck Kjeldahl trap, which is used to gather the NaOH coming with the distillate. The trap is joined to the condenser with a transport tube which dips into 50 ml of 0.1 N HCl in a conical flask, with added few drops of indicator methyl red. Added 40 % NaOH in solution till it becomes alkaline in reaction. Flask is heated and formed ammonia is let to be immersed in standard HCl. When no more ammonia was received then distillation was stopped (test with a red litmus paper turning blue). Titrate the extra of the acid with 0.1 N NaOH solution till the pink colour changes to yellow. From the titrate value calculate the multi equivalence of the acid contributing in the process of absorbing ammonia during digestion. Value is expressed as Kg/ha

2. Phosphorus (P)

It was assessed by Olsen *et al* (1954) method.

Sample Extraction

1. Weigh 2.50 g dried soil sample of 2 mm and place in a dry clean 125 ml polyethylene bottle. Run two blanks in the same method.

2. Add 50 ml of the extracting solution (0.5M sodium bicarbonate with polyacrylamide, pH 8.5) from a distributor.

3. Place bottles in a returning shaker and then shake for 30 min.

4. Filter through Whatman filter paper no. 40 into a 20 ml clean and dry test tube.

5. Estimate available phosphorus in the filtrate by colorimetry using Technicon II Autoanalyzer.

3. Potassium (K)

It was estimated by Toth *et.al.* (1948) method. In this method soil sample was acid digested in concentrated perchloric and nitric acid. The acid digest used as sample. Potassium is estimated according to standard flame photometric process by using ELICO Flame-photometer. Absorbance of flame color intensity was measured on Flame photometer with definite color filter for Potassium. Various concentrations of K+ were prepared for standardization, ranging from 1 - 10 ppm by diluting stock solution of KCl (100 ppm) from galvanometer readings. Potassium was estimated by using calibration curve of known conc of (K+). Values are expressed as Kg/ha.

4. Organic Carbon %:

Organic carbon percentage was estimated by Walkley-Black (1934) method. For sample extraction the standard wet chemistry method was used. 1.0g of the soil sample was taken in a 500 ml conical flask and added 20 ml of concentrated H_2SO_4 containing 10 ml of 0.1667 M $K_2Cr_2O_7$ and Ag_2SO_4 solution. It was then mixed carefully and finally allowed for 30 minutes to complete the reaction. This mixture was dilute with 200 ml with distilled water and 10 ml of H_3PO_4 solution and then to this 10 ml of NaF solution and then added 2 ml of diphenylamine indicator. Then this solution was titrated with standard 0.5M $FeSO_4$ solution till a brilliant green color was attained. Simultaneously, a blank without sample was also run.

The percentage of organic carbon is calculated using following formula

$$\text{Organic Carbon} = 3\ (S - T) / S$$

Where:

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

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

B. Micronutrients:

All non rhizosphere soil samples were tested analytically to their minor nutrients like Iron, Manganese, Zinc and Copper. DTPA (Diethylene Triamine Penta Acetic acid) extraction method

was followed (Lindsay and Norvell, 1978) by using Atomic Absorption Spectrophotometer (AAS) for above mentioned micronutrients analysis.

The soil samples were dried and ground to fine powder. About 5 gram of sample was taken in extraction flask. To this, 40 ml of DTPA was added using calibrated dispenser and then sample was shaken on shaker table at 200 rpm for 2 hr. After this, samples were filtered using Whatman Filter Paper No.1 and finally filtrate was used for analysis on Atomic Absorption Spectrophotometer (Perkin -Elmer - 3030).

3. 4. Biological Characteristics:

A. Collection of soil samples:

In systematic screening process for isolation of fungi, soil samples were collected during the month of Auguest-2015 to October -2018 from different localities such as Bhimashankar, Kaas-Satara, Khandala, Lawasa, Lonawala, Mahabaleshwar, Pachgani, Purandar, Aundh and Sinhagad regions of Maharashtra, India. Soil samples were collected from rhizosphere and nonrhizosphere soils of mosses. These soil samples were collected by hand uprooting mosses and shaking-off the adhering soil into a sterile polythene bags (Sule and Oyeyiola, 2012) while the non-rhizosphere soil samples were collected from soil about 1 meter away from the roots of the mosses. 500g soil samples were collected in dry, clean and sterile polythene bags by using sterilized spatula. The collected soil samples bags were brought to the laboratory and well-maintained for the studies. Fungi were isolated from the soils collected from each locality by using serial dilution techniques as described by Fawole and Oso (2007).

B) Preparation of culture media:

Potato Dextrose Agar (PDA):

Composition:

Peeled potato (200 g)

Dextrose (20 g)

Agar-agar (20 g)

Distilled water to make the final volume 1 liter, at 7 pH.

Procedure:

1) Take 500ml of sterile distilled water in one liter beaker.

2) Add 200gm.washed peels and sliced potatoes in the beaker.

3) Boil gently for 30 minutes or the time till they are easily penetrated by a glass road.

4) Filter through muslin cloth and squeeze out all liquid.

5) Add 20gm.dextrose to the potato extract.

6) Adjust the PH. of medium 6.5 -7 by using pH meter before adding agar.

7) Take 500ml of water in a new beaker and heat it.

8) Add 20gm agar and dissolve it and then mix it with the potato extract.

9) Make final volume 1 liter.

10) Plug the flask with cotton plug and sterilize in autoclave at 121^0C, 15 lbs. pressure, for 15 minutes.

11) Allow the flask to cool till the flask can be hold by hand.

12) Pour the medium in sterilized petriplates (15-20ml/plate) quickly under aseptic conditions and allow solidify, then used these plates for isolation of fungi.

C) Methods of sterilization: Media and glassware's can be sterilized by physical and chemical methods like,

1) Chemicals - Chemicals are used as cleaning solutions like alcohol, formalin, spirit etc.

2) Light - Ultra Violet light rays are used for surface sterilization.

3) Autoclave - It is used for media sterilization. Glass wares and other material can be sterilized at 15 lbs. pressure for 20 minutes. The organisms are killed, media or glassware's whatever it may be sterilized.

4) Hot air oven - This method is used for sterilization of glass wares which used for this study.

Materials and glassware's first clean, and then placed at 100 °C for 4 hrs.

D) Plating and isolation:

Rhizosphere and nonrhizosphere soils of mosses were used for isolation of soil fungi by 'serial dilution' method. In this method 1 gm. soil dissolved in 9 ml sterile distilled water, after shaking well, dilutions were made serially. 1ml of this transferred to second tube containing 9 ml sterile water resulting as 0.01 dilution, the procedure repeated to yield dilution of 0.01, 0.001, 0.0001, 0.00001 or even more if necessary. 1 ml portion from each dilution pipette to a separate test tube. Concentration ranges as 10-1, 10-2, 10-3, 10-4, 10-5, likewise 1:10, 1:100, 1:1000 and 1: 10,000. 1ml suspension from each test tube was added to sterile petriplates containing sterile Potato Dextrose Agar medium by using spread plate method (Fawole and Oso, 2007). These plates were kept in incubation chamber for 5-7 days at 28 °C. During the incubation period every 2 days of interval observed the fungal colonies in the plates. A larger number of species was isolated most of the fungus sporulate extremely. The occurrence of each fungal species was noted visually and recorded.

E) Isolation of pure culture:

Isolation of fungi carried out from incubated plates and individual organisms can multiplies to form single separate colonies on media. Test tubes containing fresh agar slants of PDA medium are used to obtain pure culture. The pure isolates obtained were then transferred onto sterile PDA slants and stored in the refrigerator at 4 to 8oC prior to their identification (Fawole and Oso, 2007).

F) Staining of Fungi:

The fungal hyphae are either colourless (hyaline) or having different colours. The hyaline mycelia, spores or conidia can be stained by cotton blue and mounted in Lactophenol.

G) Identification of Fungi:

The fungal isolates were identified using standard methods described by Fawole and Oso (2007). This was done by matching the microscopic and macroscopic features of each fungal isolate with those of known species described in relevant standard textbooks and journals. Slides

observed under light microscope. With the help of binocular microscope photographs of fungal colonies in incubated plates and microscopic view of fungi were also taken.

Fungi were identified and classified by using classification system of Ainsworth's (Ainsworth 1973, Alexopoulos *et, al*, 2002). The isolated fungi were identified up to the genus and species level on the basis of major morphological characters like the colonies slow or fast growth, heaped, flat, irregularly or regularly folded, texture of colonies like powdery, granular, or cottony, surface pigments and minor morphological characters like hyphae, conidia and other special fungal organization. The identified fungi were confirmed by microbial experts and with the help of standard books of fungi like Handbook of Soil fungi by using classification system (Nagamani *et al*. 2006)

Key for identification of fungi

Key to kingdoms

Eukaryotic organisms without plasmids, mode of nutrition absorptive, cell wall having chitin and B- glucan, generally chitinosic, motile phase when present lacking Mastigonemes……...Fungi

Kingdom fungi

1)Motile zoospore present…….....Chytridiomycota.

2) Motile zoospores absent ……1

1a. Mitospores endogenous formed in sporangia (or asexual spores derived from Sporangia) zygospores formed by hyphal conjugation ……Zygomycota.

1b. Only mitospore and vegetative propagules present ...……..…….Anamorphic fungi.

3) Mitospores exogenous, meiospore present ……2

2a .Meiospores endogenous, produced in asci ……Ascomycotaca.

2b. Meiospores exogenous formed on basidia …….....Basidiomycota.

Key to Genera

Mucaraceae

- Sporangiophores unbranched, rhizoids opposite, Sporangiophores and sporangia apophysate ……*Rhizopus.*
- Conidiophores ending into swollen vesicles bearing phialides……*Aspergillus.*
- Conidia globose, ovoid, rarely bacillar in elongated chains, borne on phialides in Brush like head (Penicillus) phialides never cylindroidal…….*Penicillium*
- Conidia hyline or lightly pigmented and macroconidia present fusiform or cylindrical, macroconidia septate with lowest cell modified to form foot cell.…… *Fusarium*
- Conidiophore branched like conifer; conidia in balls, white, yellow-green or bright green…….*Trichoderma*
- Spores formed in sporocarps in an orderly manner around a sterile central plexus. A new hypha emerges through the lumen of spore……*Glomus*

Key to species

1) *Aspergillus-*

Conidia typically green or rarely light brown, conidial crowns radiate to columnar, conidiophores smooth, vesicles dome like or subglobose, conidia elliptical, ovate or barrel shaped, globose to subglobose, roughened, rarely smooth; cleistothecia present or absent, osmophilic.

Subgenus Fumigati

Cleistothecia absent; colonies blue-green; conidia globose, echinulate……..*Aspergillus fumigatus*.

Subgenus Circumdati

Conidial heads globose to radiate, mostly loosely columnar, vesicles globose, subglobose or somewhat elongated, conidiophores may not be bound below the vesicle, uni or biseriate, scleritia commonly present.

Key

Conidial crowns radiate or very loosely columnar\, dark yellow colouered; phialides predominately biseriate……..*Aspergillus flavus*

Conidial heads dark black; conidiophores up to 3 mm in length.

Subgenus Nidulantes

Conidial colour variable, heads columnar or radiate, never black or brown, rarely white; conidiophores smooth or rough, colourless or brown; conidia globose rarely elliptical, smooth or rough; submerged vegetative mycelium sometime produces heavy walled hyaline cells.

Key

Convex walls of ascospore smooth……1

1a. Sterile spicular hyphae long, unevenly roughened; ascospores with two low equatorial apices……*Aspergillus unguis.*

Subgenus *Nigri*

Conidial heads radiate or globose or splitting into columns, black coloured; vevicle globose, nearly hyaline to brown; conidia variously shaped, smooth or rough, usually in shades of black; brown to black.

Key

Conidiophores up to 3 mm in length; conidial crania carbon black

coloured……*Aspergillus niger*

2) *Penicillium*-

Conidia borne as spheroids or ellipsoids and remaining as it is at maturity; one or two rami produced, conidia borne terminally……..…….……..*Penicillium*

Series Viridicata

Conidia grayish-blue coloured 3.5 - 4 X, 2.5 -3.2 µm; phialides 9 -10X, 2.5 -2.8 µm……*Penicillium aurantiogriseum*

Conidia blue or blue green; colonies lightly floccose; Stipes smooth walled; penicillin predominantly terverticillate……..…….*penicillium chrysogenum*

Series Italica

Conidia green, elliptical to short cylindrical, 3-4 µm, colonies velutinous to fasiculate, sometime minute coremial at the margins..…… *Penicillium italicum*

3) *Fusarium*

Colonies usually fast growing, light to bright coloured; mycelium aerial or diffuse, fruit body when present a sporodochium; sporogenous cells aries directly from vegetative to form conidiophores; conidia are of two types, macroconidia large, several septate, hyline, fusiform, cylindric, curved, microconidia smaller, non septate or one septate, in chains or in spore balls;

Key

Colonies white or peach, usually with a purple or reddish tinge; conidiophores branched or sparsely branched; microconidia formed from short lateral phialides, macroconidia, spindle to fusiform, pointed at both ends Chlamydospores mostly terminal……...*Fusarium oxysporum*

4) *Rhizopus*

Colonies fast growing 1-2 mm high, pale gray, dark brown-gray reddish brown coloured stolons, pigmented rhizoids and sporangiophores, apophysate, columellate many spored sporangia present.

Key

Rhizoids well developed, Sporangiophores irregular, round to oval, angular, straight, gray, striate 9-12 X, 7.5-8.1 µm ……*Rhizopus stolonifer.*

5) *Trichoderma*

Conidiophores branching, simple; plialides solitary particularly near the apex of conidiophores and branches, usually with intercalary phialides. Colonies with compact conidial are as, pustules spreading; conidiation yellow green to dark olive in age conidia mostly smaller than 3.5 X 2 µm ..……*Trichoderma citrino*

6) *Glomus*

Spores formed in sporocarps in an orderly manner around a sterile central plexus. A new Hypha emerges through the lumen of spore. Possesses a complete endospore formed by a more or less flexible inner wall group…….*Glomus maculosum*

3.5. Antimicrobial Screening of Mosses:

3.5.1. Preparation of mosses extract:

Extracts of the identified moss species like *Bryum ghatens* Broth. et Dix, *Hypnum reflexum* F. E. Tripp, *Steeriophyllum ancens* (Bosch et Lac.) Broth, *Fissidens crenulatus* Mitt, *Trachypodiopsis blanda* (Mitt.) Fleisch, *Funaria hygrometrica* Hedw, *Macromitrium sulcatum* Brid, *Brachymenium turgidum* Broth, *Bryum coronatum* Schwaegr, and *Hyophila involute* (Hook.) Jaeg was prepared. For this fresh plant material was washed in tap water and then in distilled water and dried on filter paper. Afterwards extraction made within four days. Plant

material (10 g) was dried at room temperature in open air and grinded in mortar and pestle with equal amount of acetone, ethanol, petroleum ether and distilled water separately. Finally, filtrates were used to screen antifungal and antibacterial activities by applying extracts saturated discs.

3.5.2. Test microorganisms and their maintenance:

All eight microbial strains are tested for their response to mosses extracts. They are obtained from National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute Pune, India. These included four bacterial strains viz. *Bacillus substilis* (NFCCI 2697), *Escherichia coli* (NFCCI 2067), *Pseudomonas aeruginosa* (NFCCI 2200), *Staphylococcus aureus* (NFCCI 2492) and four fungal strains viz. *Aspergillus niger* (NFCCI 3114), *Fusarium oxysporum* (NFCCI 1276), *Penicillium notatum* (NFCCI 1072), and *Trichoderma viride* (NFCCI 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 passable density.

3.5.3. Preparation of media for antimicrobial screening:

Nutrient agar media (NA) and Muller-Hinton agar media (MHA) are used.

Chemicals: Organic solvents like acetone, ethanol, and petroleum ether.

Antibiotics like Ampicillin and Nastatin and Sterilized distilled water.

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

A) Nutrient Agar (NA) media preparation:

Nutrient agar (NA) media is prepared, sterilized and poured 20 ml into each sterilized petri plates. 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:

Muller-Hinton agar media is prepared, sterilized and poured into sterilized petri dishes, each containing 20 ml. After plates have solidified, then, 0.2 ml suspension of organism spread on all over the plates by using glass spreader.

3.5.4. Preparation of inoculums

All bacterial strains were plated out on nutrient agar plates and incubated for 24 hr at 37ºC and colonies from this fresh culture were used for making suspension. Inoculum were prepared by transferring morphologically similar colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland, thus standard inoculum is adjusted to contain approximately 108 cfu/mL for bacteria and 107 cfu/mL for fungi. The inoculum turbidity was matched with standard 0.5 Mc Farland (Altuner, 2010).

3.5.5. 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 Nastatin, become 20 µ/ml. Same procedure was used for Ampicillin .The whole thing is done aseptically.

3.5.6. Determination of antimicrobial activity:

A. Disc diffusion assay:

Agar disk-diffusion testing developed in1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Disk diffusion test was performed as described previously by Andrews (2003). The disk diffusion assay method is simple and practical and has been well-standardized. The plant extracts were tested for antifungal and antibacterial activities through the disc diffusion method, according to the National Committee for Clinical Laboratory Standards (NCCLS, 1999, 2000, Santra *et al*, 1999; Veljic *et al*, 2009). Bauer *et al* (1966) standardized single-disk method for antibiotic susceptibility testing and update earlier descriptions of the method. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and fungi testing. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test organisms and then the diameters of inhibition growth zones

are measured. Many microbiologists concluded that finally a disk diffusion method had given good results and been used easily.

Nutrient Agar (NA) media and Muller-Hinton Agar (MHA) sterilized at 50°C and cooled for 4°C, were distributed in sterilized petri dishes. The culture medium was poured into 120 mm sterile Petri dish to give a mean depth of 4.0 mm ± 0.5 mm (Altuner and Cetin, 2009; Altuner and Akata, 2010). The Whatman No: 1 filter paper discs (6 mm in diameter) were separately saturated with 10 ml of the extract solutions and then placed on the agar plates, which had been earlier inoculated with tested microorganisms (100 µl). This suspension contained 10^6 colony forming units /ml for bacteria and 10^6 CFU/ml spores for fungal strains. Plates inoculated with bacteria were incubated at 37°C for 24 h and 30°C for 48 h for the fungal strains. The antibiotics Ampicillin (10 µg) used as positive controls for bacteria and Nastatin (10 µg) used for fungi.

After incubation nearest inhibition zone around the discs was measured in mm and used to express the antimicrobial activity. The test was done in triplicates and the mean value of the zone of inhibition was calculated. The advantages of the disk method are the test easiness that does not require any special equipment's, the provision of categorical results simply understood by all clinicians, and flexibility in selection of disks for testing. It is the least costly of all susceptibility methods. The diameter of zone inhibition is directly proportional to strength of antibiotics or bioactive extracts as well as sensitivity of microbes.

3.6. Isolation of metabolites.

3.6.1. Qualitative and quantitative analysis:

Preliminary screening of secondary metabolites: The collected mosses were washed 4-5 times in tap water for removal of other plant portions and soil debris. After washing, this material was spread on paper in room for drying. After drying it was used for extract preparation. The plant material weighted was grinded in mortar and pestle with equal amount methanol till the formation of fine paste and left for overnight then it was filtered. This filtrate was used as (100%) crude extract. The freshly prepared extract was subjected to standard phytochemical investigation to check the presence of phytochemicals such as glycosides, terpenoids, alkaloids, tannins, saponins, phenols, flavonoids and steroids by standard procedures (Horborne, 1998).

The tests were totally based on the observation of color change after the addition of specific reagents as shown in Table No.21

3.6.2. Chromatographic analysis of moss secondary metabolites.

A) Preparation of Extract for Chromatography

1. The processed 1gm plant material was weighted and grinded in mortar and pestle with 10 ml HPLC grade methanol till the formation of fine paste and left for overnight.
2. Then it was filtered by using Whatman filter paper no.40
3. This filtrate was centrifuged.
4. From supernant 0.1ml is taken and 1ml methanol is added. Then it was used for HPLC and GC-MS.

B) High-performance liquid chromatography (HPLC).

High pressure liquid chromatography, (HPLC) is a particular method of column Chromatography. This method is applicable for compounds which are soluble in solvents. This HPLC is useful for compounds that cannot be decompose or that vaporized under high temperatures. HPLC provides both quantitative and qualitative analysis in a single operation. HPLC Analysis was done for the identification of particular metabolite from methanolic extract of *Bryum coronatum.* For identification of quercetin present in the moss, standard sample solution of quercetin was run along with the plant extracts, the peak of the analyte was confirmed by comparing its retention time with that of reference standard. All the HPLC experiments were performed at T. C. College Baramati. Dist. Pune.

Methanolic extract of *Bryum coronatum* was analyzed for the presence of secondary metabolites using a Shimadzu LC 20 AR, 25 C by reverse phase. The detector was PDA and column length225cm. The analysis was carried out isodimetrically in methanol and water as 1:9 ratio. The run time for analysis was 30 minutes. Sample extract was analyzed at three different wave length i.e. 254nm, 280 nm and 330nm.

C) Gas Chromatography- Mass Spectroscopy (GCMS).

Chromatography generally used in biochemistry for separate, identify, quantify and analysis of active compounds. Qualitative and quantitative analysis of phytochemicals can be done using Gas Chromatography- Mass Spectroscopy (GCMS). GC-MS can be applied to solid, liquid and gaseous samples. In this method gas phase is flowing and the liquid phase is stationary. First the samples are converted into gaseous state then analysis is carried out on the basis of mass to charge ratio. Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds, to quantify known compounds and to elucidate the structure and chemical properties of molecules. The molecular weight of sample can be determined from MS Spectrum.The methanolic extract obtained from *Bryum coronatum,* was subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds.

Method used for GC-MS.

GC-MS analysis of the sample was carried out using Shimadzu Make QP-2010 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 50.0 °C and held for 5- 8 min and the final temperature of the oven was 280.00 °C. A 2-µL sample was injected with split mode. Plunger speed (Injection) and syringe insertion speed was high but Injection mode was normal Detector gain mode was relative to the tuning result and detector gain was1.07kV+0.00kV. Pressure was117.6kPa.Total flow was 25.0 mL/min. Column Flow was2.00 mL/min Mass spectra was recorded over 35 - 650 amu range with electron impact ionization energy 70 eV.

The chemical components from the methanolic extract of plant was identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3.7. Characterization of metabolites

3.7.1 Spectroscopic characterization of studied moss.

A. Identification of Chemical Constituents by FT-IR Spectroscopy

Spectroscopy is used to determine the functional group present in the sample. FT-IR spectroscopy used for determination of moss chemical composition. Fourier transform infrared spectra were obtained using a FTIR spectrometer Spectrum BX (Perkin-Elmer Instruments) in KBr pellets. The sample pellets used were prepared by pressing 30 mg of mixture. The mixture contained 5 mg of moss sample and 200 mg of infrared-grade KBr. FT-IR absorption spectra of moss extract was registered on a micro plate reader HTS-XT (Bruker, Germany). 50 - 330 µl of each sample were dried on a 96- place silicon plate at <50˚C. Spectra were collected over the wave-number range of 4000 - 600 cm–1, 32 scans, and resolution 4 cm–1. Data were processed with OPUS 6.0 (Bruker, Germany) software. Spectra were Vector normalized and baseline corrected by the rubber-band method. To assess and compare the FTIR spectra major sorption line intensity between different functional groups and moss species, the hydroxyl group was accepted as one unit, and another major group's absorption intensity was calculated as a ratio to the hydroxyl group.

Only some studies focus on methods used for structural characterization of organic constituents for example analytical pyrolysis-gas chromatography (py-Gc/MS) and Fourier transform infrared spectrometry (FTIR) (Kracht and Gleixner, 2000).

3.7.2 Characterization by using parameters.

The extracts have been subjected to characterize the antimicrobial properties by sensitivity of strains, by effect of pH and temperature. Following moss was selected: ***Bryum coronatum*** Schwaegr.

Parameters for Characterization:

A. Susceptibility of organisms.

B. Effect of pH.

C. Effect of temperature.

A. Susceptibility of target organisms to fresh and stored extracts of *Bryum coronatum* Schwaegr

In antimicrobial screening, Whatman No.1 filter paper discs (6 mm) are separately impregnated with 10 ml of stored and fresh extracts separately and then kept on to the agar plates, previously inoculated with test 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 1999, 2000). The antibiotics, ampicillin and Nastatin serve as a positive control.

B. Effect of pH on stability and activity of bioactive components from *Bryum coronatum* Schwaegr extract:

To decide the effect of pH on stability of bioactive components. 100 ml of bioactive extract was mixed with 1 ml of 0.1 N phosphate buffer of varied pH in different tubes, incubated for one hour at 30°C and residual antifungal activity in each tube was determined against target organisms such as *A. niger, P. notatum, F. oxysporum, and T. viride.*

C. Thermal stability of antibiotic components of *Bryum coronatum* Schwaegr extracts:

To decide the effect of temperature on stability of bioactive components screw capped ampoules, each with 100 ml 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 antifungal activity was determined against target organisms (Mansour, 2005).

4. Results and Discussion

4.1 Habit analysis:

The present study is based on the mosses collected from various localities such as Bhimashankar, Kaas (Satara), Khandala, Lawasa, Lonawala, Mahabaleshwar, Pachgani, Purandar, Aundh (Satara) and Sinhagad regions of Western Ghats of Maharashtra, India. Sixteen types of mosses were collected from the above localities. All mosses were observed and collected during the rainy season. Most of the species grow in rainy season due to high moisture

percentage in atmosphere. The mosses grow on different habitats such as tree trunks, bark of branches, on moist soils, rock surfaces, plastered and unplastered house walls. There are three terrestrial species like *Bryum ghatens* Broth. ex Dix., *Bryum coronatum* Schwager and *Hyophila involute*(Hook) Jaeg, which grows on moist soils. *Funaria hygrometrica* Hedw, grow on moist rocks. Six epiphytic species such as *Macromitrium sulcatum* Brid, *Brachymenium turgidum* Broth, *Stereophyllum ancens* (Bosch et Lac.) Broth, *Hypnum reflexum* F. E. Tripp, *Fissidens crenulatus* Mitt, *Trachypodiopsis blanda* (Mitt.) Fleisch grow on bark of tree trunk and branches. The mosses was collected during the period of July, 2015 to September, 2018 from shady places of the above localities.

This study gives idea about 10 moss species collected from 10 localities of Maharashtra. *Bryum ghatens* occurs in Khandala, Lonawala and Mahabaleshwar. *Hypnum reflexum* found in Kaas; Satara, Lonawala, Mahabaleshwar, Pachgani, Purandar, Aundh and Sinhagad. *Stereophyllum ancens* occurs at Bhimashankar, Khandala, Lonawala, Mahabaleshwar and Aundh. *Fissidens crenulatus* found at Bhimashankar, Kaas; Satara, Lawasa, Lonawala, Mahabaleshwar, Pachgani, Purandar, Aundh and Sinhagad. *Trachypodiopsis blanda* occurs in Lonawala, Mahabaleshwar, and Sinhagad. *Funaria hygrometrica* occurs at all localities. *Macromitrium sulcatum* occurs at Bhimashankar, Khandala, Lonawala, Mahabaleshwar and Purandar. *Bryum coronatum* mostly occurs at all localities except Aundh. *Brachymenium turgidum* found only at Bhimashankar, Lonawala and Mahabaleshwar. *Hyophila involute* occurs at Kaas; Satara, Khandala, Lawasa, Lonawala and Mahabaleshwar.

4.2 Collection and Identification of mosses:

Identification of mosses was performed using previous mosses 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, Dist. Pune. From collected mosses 10 species were selected for biological activities and isolation and characterization of metabolites.

The identified mosses from different localities are as

Locality: Bhimashankar

1. *Steeriophyllum ancens* (Bosch et Lac.) Broth.
2. *Fissidens crenulatus* Mitt.
3. *Funaria hygrometrica* Hedw.
4. *Macromitrium sulcatum* (Hook) Bird
5. *Brachymenium turgidum* Broth.
6. *Bryum coronatum* Schwaegr
7. *Archidium indicum* Mull.Hal
8. *Pogonatum spp.*

Locality: Kaas; Satara

1. *Hypnum reflexum* F. E. Tripp.
2. *Fissidens crenulatus* M
3. *Funaria hygrometrica* Hedw.
4. *Bryum coronatum* Schwaegr
5. *Hyophila involute* (Hook.) Jaeg.
6. *Bryum argenatum* Hedw

Locality: Khandala

1. *Bryum ghatens* Broth. et Dix
2. *Steeriophyllum ancens* (Bosch et Lac.) Broth
3. *Funaria hygrometrica* Hedw.
4. *Macromitrium sulcatum* Brid.
5. *Bryum coronatum* Schwaegr
6. *Hyophila involute* (Hook.) Jaeg.
7. *Bryum argenatum* Hewd

Locality: Lawasa

1. *Fissidens crenulatus* Mitt.

2. *Funaria hygrometrica* Hedw.

3. *Bryum coronatum* Schwaegr

4. *Hyophila involute* (Hook.) Jaeg.

5. *Bryum argenatum* Hewd

6. *Fissidens bryoides* Hedw.

Locality: Lonawala

1. *Bryum ghatens* Broth. et Dix.
2. *Hypnum reflexum F. E. Tripp.*
3. *Steeriophyllum ancens* (Bosch et Lac.) Broth.
4. *Fissidens crenulatus* Mitt.
5. *Trachypodiopsis blanda* (Mitt.) Fleisch.
6. *Funaria hygrometrica* Hedw.
7. *Macromitrium sulcatum* Brid.
8. *Brachymenium turgidum* Broth.
9. *Bryum coronatum* Schwaegr
10. *Hyophila involute* (Hook.) Jaeg.
11. *Archidium indicum* Mull.Hal
12. *Bryum argenatum* Hewd
13. *Philontis hastate* (Duby) Wijk
14. *Fissidens bryoides* Hedw.

Locality: Mahabaleshwar

1. *Brachymenium turgidum* Broth.
2. *Bryum coronatum* Schwaegr
3. *Bryum ghatens* Broth. et Dix.
4. *Philontis revolute* Bocsh et Lac
5. *Hypnum reflexum* F. E. Tripp.

6. *Steeriophyllum ancens* (Bosch et Lac.) Broth.
7. *Fissidens crenulatus* Mitt.
8. *Trachypodiopsis blanda* (Mitt.) Fleisch.
9. *Funaria hygrometrica* Hedw.
10. *Macromitrium sulcatum* Brid.
11. *Brachymenium turgidum* Broth.
12. *Philontis hastate* (Duby)
13. *Hyophila involute* (Hook.) Jaeg.

Locality: Pachgani

1. *Philontis revolute* Bosch et Lac
2. *Funaria hygrometrica* Hedw.
3. *Fissidens crenulatus* Mitt.
4. *Bryum coronatum* Schwaegr
5. *Hypnum reflexum* F. E. Tripp.

Locality: Purandar

1. *Philontis hastate* (Duby)
2. *Hypnum reflexum* F. E. Tripp.
3. *Funaria hygrometrica* Hedw.
4. *Fissidens crenulatus* Mitt.
5. *Macromitrium sulcatum* Brid.

Locality: Aundh

1. *Funaria hygrometrica* Hedw.
2. *Hypnum reflexum* F. E. Tripp.
3. *Bryum coronatum* Schwaegr
4. *Fissidens crenulatus* Mitt
5. *Steeriophyllum ancens* (Bosch et Lac.) Broth.

Locality: Sinhagad

1. *Hypnum reflexum* F. E. Tripp.

2. *Fissidens crenulatus* Mitt.
3. *Trachypodiopsis blanda* (Mitt.) Fleisch
4. *Funaria hygrometrica* Hedw.
5. *Bryum coronatum* Schwaegr
6. *Hydrogonium arcuatum*

Following 10 species were selected for studies:

1. *Bryum ghatens* Broth. et Dix.
2. *Bryum coronatum* Schwaegr
3. *Hypnum reflexum* F. E. Tripp.
4. *Steeriophyllum ancens* (Bosch et Lac.) Broth.
5. *Fissidens crenulatus* Mitt.
6. *Trachypodiopsis blanda* (Mitt.) Fleisch.
7. *Funaria hygrometrica* Hedw.
8. *Macromitrium sulcatum* Brid.
9. *Brachymenium turgidum* Broth.
10. *Hyophila involute* (Hook.) Jaeg.

Table 1: Number of moss species collected from each locality of Maharashtra.

Sr. No.	Name of collected species	Localities									
		A	B	C	D	E	F	G	H	I	J
1	*Bryum ghatens Broth.* et Dix.	-	-	+	-	+	+	-	-	-	-
2	*Hypnum reflexum* F. E. Tripp.	-	+	-	-	+	+	+	+	+	+
3	*Steeriophyllum ancens* (Bosch et Lac.) Broth.	+		+		+	+	-	-	+	-
4	*Fissidens crenulatus* Mitt.	+	+		+	+	+	+	+	+	+
5	*Trachypodiopsis blanda* (Mitt.) Fleisch.	-	-	-	-	+	+	-	-	-	+
6	*Funaria hygrometrica* Hedw.	+	+	+	+	+	+	+	+	+	+
7	*Macromitrium sulcatum* Brid.	+	-	+	-	+	+	-	+	-	-

8	*Brachymenium turgidum* Broth.	+	-	-	-	+	+	-	-	-	-
9	*Bryum coronatum* Schwaegr	+	+	+	+	+	+	+	-	+	+
10	*Hyophila involute* (Hook.) Jaeg.	-	+	+	+	+	+	-	-	-	-
11	*Archidium indicum* Mull. Hal	+	-	-	-	-	+	-	-	-	-
12	*Bryum argenatum* Hewd	-	+	+	+	+	+	-	-	-	-
13	*Philontis hastate* Duby.	-	-	-	-	+	+	+	+	-	-
14	*Fissidens bryoides* Hedw	-	-	-	+	+	-	-	-	-	-
15	*Pogonatum oloids*	+	-	-	-	+	-	-	-	-	-
16	*Hydrogonium arcuatum*	-	-	-	-	-	-	-	-	-	+
	Total	8	6	7	6	14	13	5	5	5	5

Present (+), Absent (-)

Localities: A-Bhimashankar, B- Kaas; Satara, C-Khandala, D- Lawasa, E- Lonawala, F-Mahabaleshwar, G-Pachgani, H- Purandar, I-Aundh and J-Sinhagad.

Table 2: List of mosses collected from different Habitats

	Order	**Family**	**Species**	**Habitat**
1	Polytrichales	Funariaceae	*Funaria hygrometrica* Hedw	Lithophytic
2	Bryales	Orthotrichaceae	*Macromitrium sulcatum* Brid.	Epiphytic on bark of tree
3	Eubryales	Bryaceae	*Brachymenium turgidum* Broth.	Epiphytic on bark of tree
4	Eubryales	Bryaceae	*Bryum ghatens* Broth. ex Dix.	Terrestrial
5	Eubryales	Bryaceae	*Bryum coronatum* Schwager	Terrestrial
6	Hypnobryales	Thuidiaceae	*Stereophyllum ancens*	Epiphytic on tree

			(Bosch et Lac.) Broth	branches
7	Hypnobryales	Hypnaceae	*Hypnum reflexum* F. E. Tripp.	Epiphytic on tree
8	Pottiales	Pottiaceae	*Hyophila involute(*Hook) Jaeg	Terrestrial
9	Fissidentales	Fissidentaceae	*Fissidens crenulatus* Mitt.	Epiphytic on bark of stem
10	Isobryales	Trachypodaceae	*Trachypodiopsis blanda* (Mitt.) Fleisch	Epiphytic on branches

4.3 Physico-chemical characteristics of mosses associated soils:

Soil is composed of mineral matter together with trace amount of organic matter, addition with various microorganisms. It is a base of greater chemical and biological activities. Soil microbes play an important role in decomposition of organic matter and nutrient cycling. Physical characteristics of mosses associated soils are studied for their colour, texture and structure analysis.

4.3.1 Physical characteristics:

A. Soil colour:

Soil colour was observed on the spot at the time of collection. It shows variations, laterite soil at Kass (Satara), red soil from Lonawala, light black soil from Khandala, reddish brown at Sinhagad, Yellow brown at Mahabaleshwar and Pachgani. Brown and light brown soil samples observed at Aundh and Bhimashankar (**Table. 3**).Multi- colours of soils are due to phenomenon of oxidation and reduction of iron and manganese compounds and their hydrated

products. Colours are an indicator of geographical and pedological origin of the soil. Soil science deals with the aspects of nutrients availability, microbial activity, wetness.

B. Soil texture:

Soil texture and their results by mechanical analysis are predicted in Tables 4. The Sandy clay, Silty clay loam, Sand loamy, Clay silty, Clay silty, clay, loamy coarse sand , Clay loamy, Clay loamy, Loamy coarse sand and Sand loamy soils are reported subsequently from studied localities. . The loamy sand and deep black soil contains a very large amount of fine particles, especially from Purandar, which favours the growth of mosses.

Table. 3: Colour of soils associated with mosses.

Sr. No.	Locality	Colour of the soil particles
1	Bhimashankar	Light Brown
2	Kass (Satara)	Laterite
3	Khandala	Light Black
4	Lawasa	Deep Brown
5	Lonawala	Red Sandy Soil
6	Mahabaleshwar	Yellow brown
7	Pachgani	Yellow brown
8	Purandar	Deep black

9	Aundh	Brown
10	Sinhagad	Reddish brown

Table 4: Texture of soils associated with mosses (in %).

Sr. No.	Locality	Layer				
		Clay	Silt	Fine sand	Course sand	Textural class
1.	Bhimashankar	30	15	10	45	Sandy clay
2.	Kaas-Satara	28	22	35	15	Loamy sand
3.	Khandala	30	18	27	25	Clay silty
4.	Lawasa	35	15	25	25	Coarse sand
5.	Lonawala	42	13	10	35	Clay silty
6.	Mahabaleshwar	37	23	18	22	Loamy coarse sand

7.	Pachgani	35	25	16	24	Loamy coarse sand
8.	Purandar	30	16	20	34	Sand loamy
9.	Aundh	30	20	25	25	Loamy coarse sand
10.	Sinhagad	30	33	22	15	Clay silty loamy

C. pH of soil:

Soil pH ranges from 5.5 to 7.35. The pH values of soil in Lonawala and Mahabaleshwar was alkaline (7.0 >7.5). In Pachgani, Purandar and Sinhagad pH was medium acidic (5.5 > 6.0).The pH was slightly acidic (6.0 > 6.5) in Bhimashankar, Kass (Satara), Khandala and Lawasa. Therefore most of the localities are acidic only two localities are alkaline in nature. pH means (H+) concentration. Soils acidity or alkalinity depends on it (Table 5). The soils are grouped into various soil natures by changing their pH range analysis is shown in Table 5 (Winston, 1968).

Bryum coronatum and *Funaria hygrometrica* mostly grows on alkaline soils (Ikenberry.1936) he find out *Bryum coronatum* and *Funaria hygrometrica* as indicators of alkaline or neutral soils. The above information suggest that the distribution of mosses not only depends on pH alone but also other factors like moisture content and mineral elements plays important role in it. Our results of pH analysis from different localities could differ and shows the flexible pH ranges.

The pH differences mostly reveal the difference in uptake ability of calcium ions and several mineral nutrients. From alkaline and neutral soils, some thalloids prefer acidic and some prefer calcareous soil. Bryophytes are sensitive to pH and water availability and would perhaps respond to factors associated with tree species.

E. Electrical conductivity (EC):

Electrical conductivity of soil of different localities are recorded in Table 5. It was found that EC was ranging from 0.19 to 6.29 dS/m .EC is measured in units called deciseimens per meter (e.g. dS/m).EC of soil indicates the cation exchange ability, salinity and porosity of soil. If EC value of soil solution is 4 dS m -1 or more than the soil is considered as saline. In our result only two sites are saline and remaining soils shows low EC values indicates less free ion availability which affect porosity of soil. Result showed that non-rhizosphere soil of only Lonawala soil is alkaline and soils of other localities are medium to slightly acidic.

Table 5: pH and EC analysis of non-rhizosphere soils of mosses.

Sr.No	Locality	Moss species	pH	EC dS m-1
1	Bhimashankar	*Brachymenium turgidum* Broth	6.30	0.72
2	Kaas-Satara	*Bryum coronatum* Schwaegr	5.93	0.63
3	Khandala	*Bryum ghatens Broth. et Dix*	6.29	0.65
4	Lawasa	*Funaria hygrometrica* Hedw	5.60	0.25
5	Lonawala	*Fissidens crenulatus Mitt.*	7.64	0.15
6	Mahabaleshwar	*Hyophila involuta* (Hook) Jaeg	6.4	1.35
7	Pachgani	*Hypnum reflexum F. E. Tripp.*	6.70	0.75
8	Purandar	*Macromitrium sulcatum* Brid	6.80	5.75
9	Aundh	*Steeriophyllum ancens (Bosch et Lac.) Broth.*	6.2	6.55

10	Sinhagad	*Trachypodiopsis blanda (Mitt.) Fleisch.*	6.28	6.51

Table6: Soil pH and its interpretation (Winston, 1968)

Range	Denomination
> 4.0	Vary strongly acidic
4. 0 > 5.5	Strongly acidic
5.5 > 6.0	Medium acidic
6.0 > 6.5	Slightly acidic
6.5 > 7.0	Vary slightly acidic
7.0 > 7.5	Vary slightly alkaline
7.5 > 8.0	Slightly alkaline
8.0 > 8.5	Medium alkaline
8.5 > 10	Strongly alkaline

4.3.2 Chemical characteristics of non-rhizosphere soils of mosses:

Soil is a multi-layered surface of various minerals and organic constituents present in solid states. They are mixed together by natural processes. Due to this mixing, they are gathered in to a porous soil. The mineral portion of soil results from the weathering and erosion of rock. Various soil types like, silt or clay is defined as largest to smallest of particle size. These particles may be loose, pack or having pour spaces. These pores are house of air and water. Soil contains four major components like organic matter, minerals, air and water. Soil also contains microbes, plants, animals and man-made materials. Plant roots have specific habitats for microorganisms. Soil provides nutrients, air, water and anchorage to the plants. It also plays important role in recycling of minerals and also purify water.

For present study, soil samples were collected from various localities such as Bhimashankar, Kaas-Satara, Khandala, Lawasa, Lonawala, Mahabaleshwar, Pachgani, Purandar, Aundh and Sinhagad from Maharashtra, during rainy season July, 2015 to September 2018. Soil samples were collected from shady places and wet soils of studied localities.

A. Macro nutrients analysis of non-rhizosphere soils.

The mosses associated soil analysis from each localities are shown in Table 7. The result indicate that available Nitrogen, Phosphorous, Potassium and Carbon %, ranges from 142.2 to 561 %, 1.34 to16.90, 114.10 to 472, 0.23 to3.91 Kg/ha respectively.

1. Nitrogen

Nitrogen is one of the widely distributed major element in the nature. Its maximum amount is present in a fixed form in the earth crusts sediments and rocks. It is a major component of proteins, amino acids and nucleic acids. It is a constituent of enzymes and chlorophyll. It also involved in various metabolic reactions taking place in plants life. Nitrogen content is maximum in *Trachypodiopsis blanda* and minimum in *Hyophila involute*.

2. Phosphorus

Phosphorus is second macro element, which is a major component of ATP molecules. For transport of energy out of the chloroplasts inorganic phosphate is required. Various metabolic processes directly or indirectly depend on this energy. Inadequate phosphate supply may affects processes like protein synthesis and nucleic acid synthesis. The rate of phosphate uptake decreases with increasing pH of soil. In the present investigation Phosphorus content of *Bryum ghatens* is maximum and minimum in *Macromitrium sulcatum*.

3. Potassium

Potassium is one of the important cation, which plays significant role in various physiological and biochemical processes. It is actively participated in water absorption, phosphorylation, opening and closing of stomata and diffusion of CO_2 to mesophyll. In this investigation the potassium content of *Trachypodiopsis blanda* is maximum and minimum in *Hypnum reflexum*.

4. Carbon

Carbon is taken from atmosphere. It is investigated from the present results (Table7) that the maximum amount of carbon was found in soil of *Bryum ghatens* while lowest in *Fissidens crenulatus.* Thus above result shows that non- rhizosphere soils of mosses are rich in organic matter with sufficient amount of available nitrogen, phosphorous and potassium.

Kashid and Chavan (2011) find out nutrient contents in the rhizosphere soils of some thalloids from, Western Ghats of Maharashtra. Soil samples were collected from rhizospheres of *Plagiochasma simlensis*, Kash, *Cyathodium tuberosum* Kash, *Targionia hypophylla* L, *Fossimbronia indica* St, *Riccia discolor* Kash, and *Sewardiella tuberifera* Kash. Soil samples were studied for available nitrogen, phosphorus, potassium and carbon percentage. The result obtained indicates that, the available N (561.94 Kg/ha) and K (654.08 Kg/ha) contents in rhizosphere soil of *Targionia hypophylla* was high, while it was minimum with *Fossimbronia indica* (3.5 Kg/ha). Rhizosphere of *Targionia hypophylla* showed higher available P (4.21Kg/ha), while available organic carbon was (3.37%) in *Plagiochasma simlensis*. Result showed that the soils of Western Ghats of Maharashtra are rich in nitrogen and potassium with sufficient amount of carbon, which favours the growth of bryophytes.

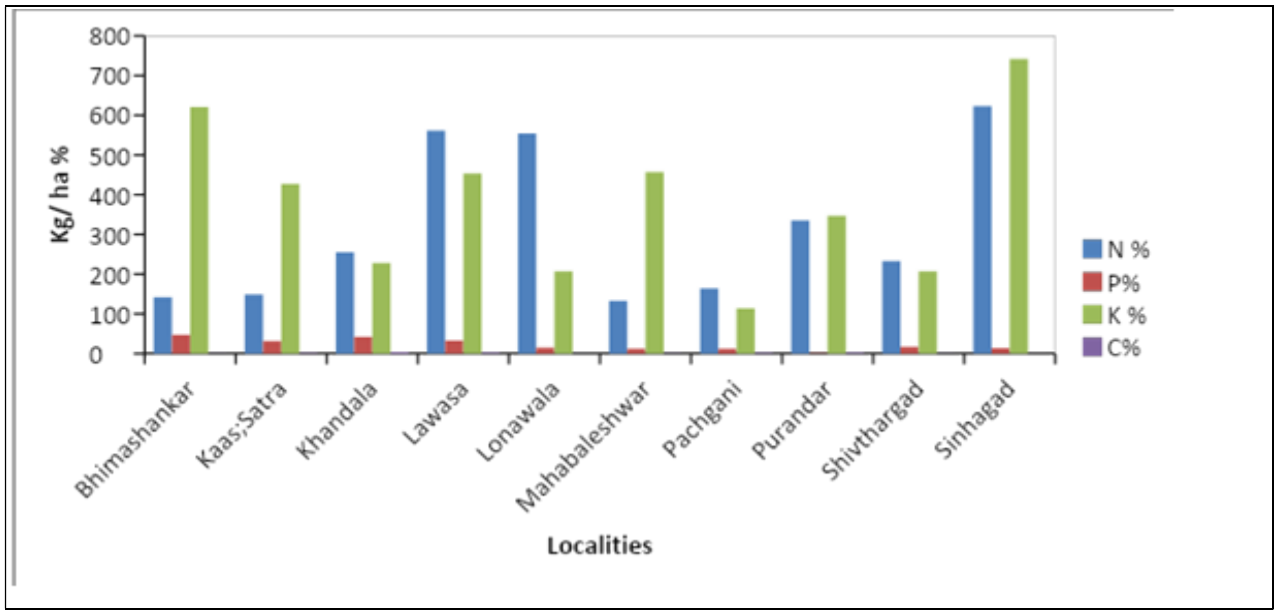
The rating of soil testing values of essential nutrients from different localities of Maharashtra are compared with ICAR (2006) rating chart of soil testing values and our observations are made for variation of low and high values. The results indicate that soil nutrients like Nitrogen (N), Phosphorous (P) and Potassium (K) percentage is higher as compare to organic matter (C). Therefore, as per ICAR (2006) rating values of essential nutrients, we summarize the findings that the K and C values are less to medium than average values at all studied localities.

Table7: Macro nutrients analysis of non-rhizosphere soils of mosses.

Sr. No.	Locality	Moss species	N %	P%	K %	C%
1.	Bhimashankar	*Brachymenium turgidum* Broth	142	47.3	620	0.61
2.	Kaas;Satra	*Bryum coronatum* Schwaegr	149	31.4	427	1.83
3.	Khandala	*Bryum ghatens Broth. et Dix*	255	**42.2**	228.21	**3.91**
4.	Lawasa	*Funaria hygrometrica* Hedw	561	32.9	453	2.73
5.	Lonawala	*Fissidens crenulatus Mitt.*	554	15.03	207	**0.23**
6.	Mahabaleshwar	*Hyophila involuta* (Hook) Jaeg	**133**	12.2	457	0.87
7.	Pachgani	*Hypnum reflexum F. E. Tripp.*	164	12.12	**114.10**	2.17
8.	Purandar	*Macromitrium sulcatum* Brid	335	**1.39**	347	2.73
9.	Aundh	*Steeriophyllum ancens (Bosch et Lac.) Broth.*	233	16.90	207	0.56
10.	Sinhagad	*Trachypodiopsis blanda (Mitt.) Fleisch.*	**623**	14.02	**742**	0.26

(Values are expressed in Kg/ha)

Fig. 1: Macro nutrients content from non-rhizosphere soils of different localities.



B. Micro nutrients from non rhizosphere soil.

All non rhizosphere soil samples are tested analytically to their minor nutrients like Iron, Manganese, Zinc and Copper. Values of nutrient elements in studied mosses soils varies from species to species. Mineral nutrition was investigated in mosses associated soils.

1. Iron:

From result Table 8 it is clear that the level of iron is the highest among all the micronutrients in the studied mosses soils (1125-1493 µg /g). Fe content is recorded more in *Hyophila involuta* (1493 µg/g) and minimum in *Bryum ghatens* (1125 µg /g). It is an important element in oxido-reduction reactions in plants, its performance justifies normal healthy growth of mosses. It is essential for synthesis of chlorophyll pigments.

2. Manganese:

Table 7 gives idea about the level of manganese in analyzed soils of mosses. There is varied range of Mn concentration ranging from 96 to 356 µg/g. In present soil analysis it is evident that the maximum amount of Mn was found in *Hyophila involuta* (335 µg/g) and minimum amount in *Bryum ghatens* (96 µg/g) as compared to other mosses. It is required for chlorophyll formation and oxide-reduction reaction of cells. Its deficiency causes chlorosis followed by necrosis. It brings the oxidation of IAA by activating IAA oxidase.

3. Zinc:

The Present result (Table 8) reported that the level of zinc ranges from 35-80 µg/g. *Bryum coronatum* shows the highest amount of zinc (35 µg/g)and minimum amount in *Funaria*

hygrometrica (85 µg/g). According to Epstein (1972) for optimal growth of plants zinc requirement is 0.02 mg/g soil. Zinc is required for chlorophyll biosynthesis and activity of many enzymes. It is involved in the synthesis of indole acetic acid. Its deficiency results in stunted growth of plants. Its toxicity results in leaf expansion, followed by chlorosis and reduction in root growth.

4. Copper:

The present result (Table8) reported that the level of copper ranges from 7-57 mg/g. *Bryum ghatens* shows the minimum amount of copper (7 µg/g) and maximum amount in *Hyophila involuta* (57 µg/g). It is a constituent of chloroplasts and plastocyanin, which are involved in electron transfer system of respiration. It is also involved in synthesis of various plant pigments and synthesis and stability of chlorophylls. It is an important element of enzymes which are involved in redox reaction of photosynthesis. It is involved in synthesis of auxins.

Table8: Micro nutrients from non rhizosphere soil. (µg/g) ppm

Sr. No	Name of moss species	Fe	Mn	Zn	Cu
1	*Brachymenium turgidum* Broth	1411	257	49	26
2	*Bryum coronatum* Schwaegr	1289	96	35	07
3	*Bryum ghatens Broth. et Dix*	1125	260	56	41
4	*Funaria hygrometrica* Hedw	1407	200	80	37
5	*Fissidens crenulatus Mitt.*	1232	321	73	26
6	*Hyophila involuta* (Hook) Jaeg	1493	356	57	57
7	*Hypnum reflexum F. E. Tripp.*	1324	270	61	38
8	*Macromitrium sulcatum* Brid	1404	198	76	35
9	*Steeriophyllum ancens (Bosch et Lac.) Broth.*	1423	235	47	46
10	*Trachypodiopsis blanda (Mitt.) Fleisch.*	1223	265	39	32

Fig 2: Micro nutrients from non rhizosphere soil of mosses.

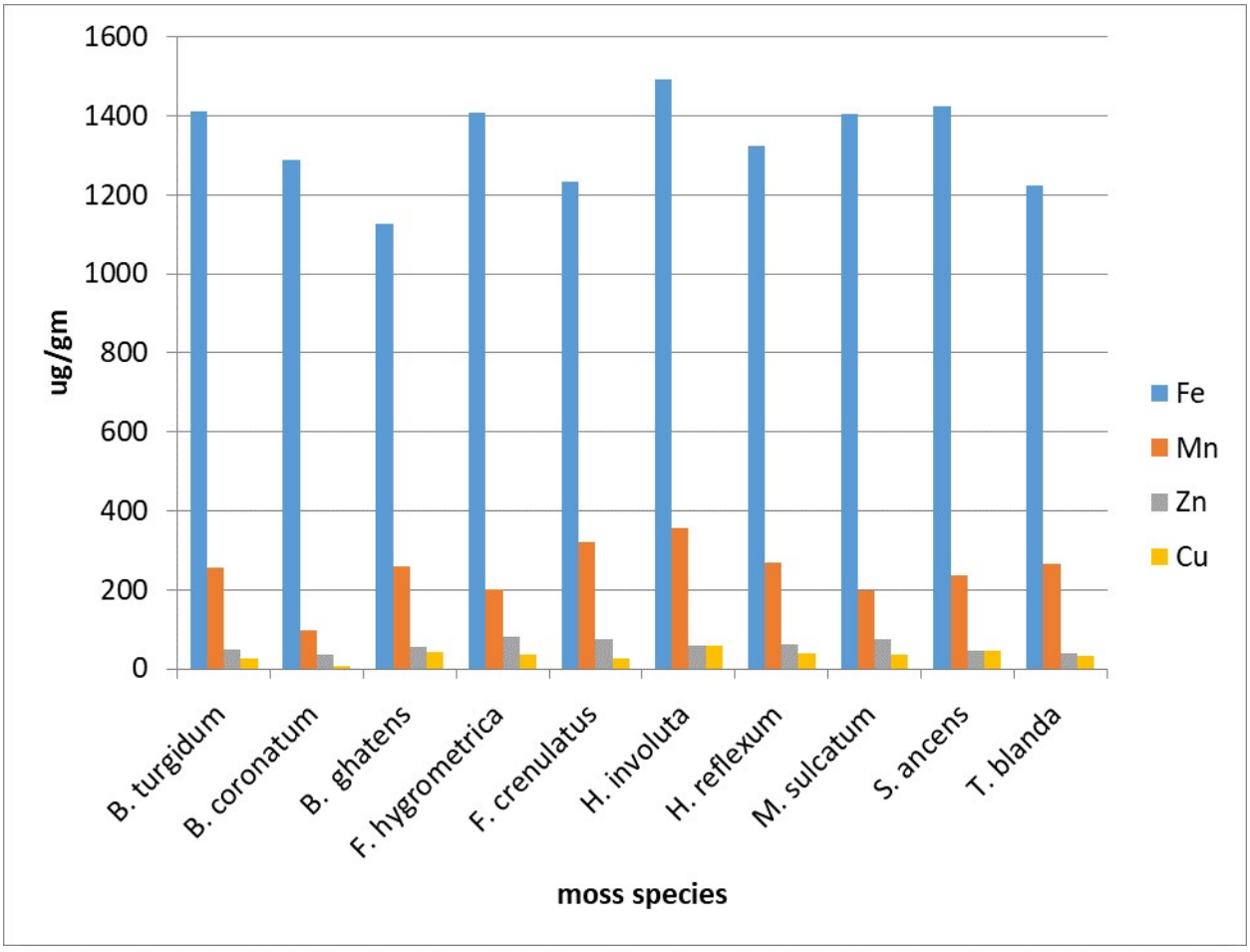


Table 9: Rating Distribution of nutrients from some moss species.

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 mole (P^+) / kg	<1.5	-	>1.5
6	Mg	Ammonium Acetate mole (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).

4.4 Biological characteristics:

4.4.1 Isolation of rhizosphere soil fungi:

Present study carried out for an effort to know the soil fungal diversity and systematic screening method for isolation of fungi. Soil samples were collected from different localities such as Bhimashankar, Kas-Satara, Khandala, Lawasa, Lonawala, Mahabaleshwar, Pachgani, Purandar, Aundh and Sinhagad regions of Maharashtra, India. The study aimed that the isolation of soil fungi from rhizosphere and non- rhizosphere soils of moss species during the period of July-September 2015-18. Mycoflora diversity in rhizosphere soil of mosses from Western Ghats of Maharashtra is depicted in table 11. During investigation 12 isolates obtained from the soil samples and were identified with standard key and microbial expert.

The identified soil fungi (Table 11) are *Aspergillus flavus, A. fumigatus, A. niger, A. unguis, Candida albicans, Fusarium oxysporum, Glomus fasciculatum, Penicillium aurantiogriseum P. chrysogenum, P. scandium, Rhizopus stolonifer, Trichoderma citrinoviride and Penicillium sp.* Among the identified species the fungus *Aspergillus niger* found maximum numbers and followed by *Penicillium* sp. Fungal species isolated from *Funaria hygrometrica* Hedw. are *Aspergillus niger, Penicillium chrysogenum, Glomus fasciculatum* and *Aspergillus unguis*. Its numbers are maximum from the isolated rhizosphere soils samples.

Macromitrium sulcatum Brid associated fungi are *Aspergillus niger, Penicillium aurantiogriseum* and *Fusarium oxysporum .Aspergillus niger, Penicillium chrysogenum* and *Glomus fasciculatum* are associated with *Brachymenium turgidum* Broth. Fungal species isolated from rhizosphere soil of *Bryum coronatum* Schwaegr are *Rhizopus stolonifer, Aspergillus flavus, penicillium islandium* and *Aspergillus fumigatus* .The species like *Aspergillus niger, Rhizopus stolonifer*, *Candida albicans, Trichoderma citrinoviride* and *Aspergillus fumigatus* are isolated from *Hyophila involuta* (Hook) Jaeg.

The species are *Aspergillus niger, Penicillium aurantiogriseum, Fusarium oxysporum and, Rhizopus stolonifer* found in *Hypnum reflexum* F. E. Tripp. The moss of *Fissidens crenulatus* Mitt. isolated fungi are *Aspergillus niger, Penicillium chrysogenum, Glomus fasciculatum* and *Aspergillus unguis*. Fungal species isolated from *Trachypodiopsis blanda (*Mitt.) Fleisch. are *Aspergillus niger, Penicillium chrysogenum, Glomus fasciculatum* and *Aspergillus unguis. Steeriophyllum ancens* (Bosch et Lac.) Broth. Rhizosphere soil fungi are

Aspergillus niger, Penicillium chrysogenum, Glomus fasciculatum, Aspergillus unguis and *Aspergillus fumigatus.*

4.4.2 Isolation of non- rhizosphere soil fungi:

During investigation 10 isolates obtained from the non- rhizosphere soil samples.

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

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

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

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

Lonawala: *Aspergillus, Saccharomyces, Mucor.*

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

Pachgani; *Aspergillus, Saccharomyces.*

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

Aundh: *Aspergillus, Penicillium.*

Sinhagad*: Aspergillus, Glomus.*

From studied localities numerous genera of fungi found in soil. Majority of fungi are saprophytic and few are parasitic. The most *common* are *Aspergillus, Saccharomyces, Fusarium, Mucor, Penicillium* and *Rhizopus.* Fungus *Aspergillus* is common and found in all studied localities. A few species belonging to genera *Trichoderma* and *Glomus* but, they are less abundant. Fungal flora varies with the nature of soil condition. There number are more in acidic soils than in alkaline or neutral soils and the number of fungi is usually low in summer, and high in monsoon (Waksman, 1944). Kamal and Singh (1970) observed rhizosphere mycoflora of some bryophytes.

Fungal flora differs with the environment of soil condition. Soil factors like soil acidity and the amount of organic matter present in it, effects the fungal population considerably. Actinomycetes and bacteria are more active in alkaline and neutral soils. Iqbal *et al.* (1988 a.) studies vesicular-arbuscular mycorrhizal fungi associated with three mosses (*Sphagnum cymbifolium, Polytrichum communae* and *Funaria hygrometrica).*

Soil factors like the amount of organic matter present in soil and acidity influences the fungal population considerably. In alkaline or neutral soils actinomycetes are more active.

A large no of references are available on association of mycorrhiza with bryophytes. In the present investigation *Glomus fasciculatum* AM fungi in rhizosphere soil of *Brachymenium*

turgidum Broth was observed. The bryophytes have never been found without fungal association (Doebbeler, 1996). He also reported about 300 species of Ascomycetes which grow as obligate parasites on bryophytes. Bryophytes are well known to form association with fungi and a few liverworts are associated with endophytes (Read *et al*, 2000).

Physical and chemical nature of soil influences the moisture, air and food supply of the microorganisms and affects the diversity and abundance of the microbial population. They indicated antagonistic effects themselves. Type of vegetation also influences the soil microorganisms (Weibull, 2000). Tapwal *et al*. (2004) noted that mycoflora of rhizosphere soil influence the growth of bryophytes.

There is a remarkable association of fungi and bryophytes but very less attention has been paid by bryologists. Russell and Bulman, (2004) reported that the Arbuscular Mycorrhiza fungus *Glomus* forming an endo-symbioses with liverwort *Marchantia foliacea*. Opelt and Berg (2004) investigated that very little is known about the interaction of bryophytes with bacteria.

From identified fungi *Aspergillus flavus* causes aspergillosis of the lungs sometimes causing corneal and naso-orbital infections. Its spores are allergenic. It is the second most common agent of aspergillosis, the first being *Aspergillus fumigatus*. *Aspergillus niger* is pathogenic and growing very quickly. It is one of the most common species of the genus *Aspergillus* and causes black mould disease on fruits and vegetables; it is also a common contaminant of food (Sharma, 2012). *A. fumigatus* is useful in recycling carbon and nitrogen from dead organisms.

Candida albicans is the most pathogenic and prevalent species of the *Candida* genus (Williams *et al.,* 2013). *Trichoderma* sp. has bio control potential against *Ganoderma boninense*.

Analysis have essentials to get alertness of threatening varied mycoflora as early as possible. There was significant variation in physical and chemical characteristics of mosses 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 mosses associated soils. These fungi are able to produce secondary metabolites which have antibiotic activity.

This study will provide model of the fungi associated with the rhizosphere and non-rhizosphere soils of mosses. The objectives of this study was to isolate and identify fungi species

in the rhizosphere and non- rhizosphere soils of mosses and to determine the biological characteristics of the soil. The rhizosphere soils studied were slightly acidic (pH 5.8). These acidity levels favors microbial establishment.

These results could support the references of mycoflora as an alternative of the host for medicinal activity. The various localities of Western Ghats of Maharashtra, have seem to affect the frequency and density of mycoflora. This isolated mycoflora varies in different localities showing variation in soil quality. This analysis yielded 12 fungal genera from mosses associated soils.

Table 10: Identification of fungal species from some moss species.

Sr. No	Name of Fungi	Colony Colour	Thallus structure	Spore diameter (µm)

1	*Aspergillus flavus*	Yellow-Green	Long stalked conidial structure greenish yellow	3.5-4.5
2	*Aspergillus niger*	Black	Black spores, mycelium submerged vesicle globose, rarely septate	4-5
3	*Aspergillus unguis*	Yellowish green	Sterile thick walled hyphae, roughened, spicular.	3.2-3.5
4	*Aspergillus fumigates*	Blue-green	Conidiophores short, smooth, light green	2.5-3
5	*Fusarium oxysporum*	White	Arial mycelium sparse to floccose Sickle shaped conidia	2-5
6	*Candida albicans*	White	Mycelium largely submerged, hyaline; pseudohyphea and true hyphae also observed	2.5-3.5
7	*Rhizopus stolonifer*	Grayish	Long stalk, mycelia fused, rhizoids present	4-6
8	*Penicillium aurantiogriseum*	Gray, dull green	Mycelium white usually inconspicuous	2.5-3.2
9	*Penicillium chrysogenum*	Brown	Mycelium margins white blue green conidia are present	2.2-3.5
10	*Trichoderma citrinoviride*	Blue green	Mycelium submerged or watery white, composed of hyaline	1.5-2.1
11	*penicillium italicum*	Blue green	Mycelium intensely colored	2.2-2.5
12	*Glomus fasciculatum*	Brown	Arbuscules forms branched structure, it also forms vesicles a swollen structure	4-6

Table 11: Fungal genera and species isolated from mosses rhizosphere soils

Sr.No	Mosses	Name of fungal genera	Total
1	*Funaria hygrometrica* Hedw	*Aspergillus niger, Penicillium chrysogenumGlomus fasciculatum*, *Aspergillus unguis.*	04

2	*Macromitrium sulcatum* Brid	*Aspergillus niger, Penicillium aurantiogriseum Fusarium oxysporum.*	03
3	*Brachymenium turgidum* Broth	*Aspergillus niger, Penicillium chrysogenum and Glomus fasciculatum.*	03
4	*Bryum coronatum* Schwaegr	*Rhizopus stolonifer, Aspergillus flavus, penicillium islandium, Aspergillus fumigatus.*	04
5	*Hyophila involuta* (Hook) Jaeg.	*Aspergillus niger, Rhizopus stolonifer*, *Candida albicans, Trichoderma citrinoviride,Aspergillus fumigatus.*	05
6	*Bryum ghatens* Broth. et Dix.	*Rhizopus stolonifer, Aspergillus flavus, penicillium islandium, Aspergillus fumigatus.*	04
7	*Hypnum reflexum* F. E. Tripp.	*Aspergillus niger, Penicillium aurantiogriseum, Fusarium oxysporum, Rhizopus stolonifer.*	04
8	*Fissidens crenulatus* Mitt.	*Aspergillus niger, Penicillium chrysogenum, Glomus fasciculatum, Aspergillus unguis.*	04
9	*Trachypodiopsis blanda (*Mitt.) Fleisch.	*Aspergillus niger, Penicillium chrysogenum, Glomus fasciculatum, Aspergillus unguis.*	04
10	*Steeriophyllum ancens* (Bosch et Lac.) Broth.	*Aspergillus niger, Penicillium chrysogenum, Glomus fasciculatum, Aspergillus unguis, Aspergillus fumigatus.*	05

4.4.3. Antimicrobial Screening of Mosses:

In presented study, the antimicrobial activity of 10 moss species collected from various localities of Western Ghats of Maharashtra was evaluated. The antimicrobial activity of aqueous, methanolic, ethanolic and petroleum ether extracts of 10 moss species was tested against four bacterial strains viz. *Bacillus substilis* (NFCCI 2697), *Escherichia coli* (NFCCI 2067), *Pseudomonas aeruginosa* (NFCCI 2200), *Staphylococcus aureus* (NFCCI 2492) and four fungal

strains viz. *Aspergillus niger* (NFCCI 3114), *Fusarium oxysporum* (NFCCI 1276), *Penicillium notatum* (NFCCI 1072), and *Trichoderma viride* (NFCCI 1139).

A. Antifungal activity

In this study, the antimicrobial effects of ten moss species, having four different solvent extract (ethanol, methanol, petroleum ether and water), were compared with standard antibiotics used as positive controls. Antifungal activity of plant extracts in different solvents on test microorganisms are given in Table.

Antifungal screening of *Bryum ghatens* and *Hypnum reflexum* methanolic extracts indicates the greater inhibitory activity against *Fusarium oxysporum* (Table 10). *Funaria hygrometrica* shows maximum zone of inhibition 1.0 mm against *Penicillium notatum* and minimum for *Hyophila involuta.* Ethanol extracts of *Bryum coronatum, Hyophila involuta* and *Hypnum reflexum* shows maximum 0.9 mm zone of inhibition against *Fusarium oxysporum* and minimum 0.4 against *Stereophyllum anceps*. (Table 11)

Petroleum ether extracts showed more antifungal activity, against *Penicillium notatum* for *Funaria hygrometrica* followed *Hyophila involuta* against *Aspergillus niger*. The no inhibition zone was occurred for *Bryum ghatens, Hypnum reflexum, Trachypodiopsis blanda, Funaria hygrometrica* and *Bryum coronatum* for all tested organism. *Brachymenium turgidum* and *Steeriophyllum ancens* does not shows inhibition zone against *A niger, P notatum* and *Trichoderma viride.*

Aqueous extract of *Hypnum reflexum* shows maximum zone of inhibition 1.0 mm for *Trichoderma viride* and minimum zone of inhibition for *Bryum coronatum* 0.2 mm (Table 13) for *A. niger* and other extracts shows moderate zone of inhibition. The inhibition zone was not seen for *Bryum ghatens, Fissidens crenulatus, Trachypodiopsis blanda* and *Steeriophyllum ancens* for *Fusarium oxysporum* respectively. We know that conventional antibiotics are generally more active against the gram positive bacteria than gram negative bacteria. However, these mosses samples showed inhibition effect against both the gram positive and negative bacteria.

Some researchers reported special antimicrobial activities of different bryophyte samples against gram-negative bacteria (Basile *et al*., 1998; Bodade *et al*., 2008). The results obtained are similar to some researcher's report that extracts from mosses displayed antifungal activities

(Castaldo-Cobianchi *et al.,* 1988; Bodade *et al.,* 2008). Kaushik *et al, (*2000) reported that *E. coli* was sensitive to the alcoholic extract of liverwort *Riccia*.

Subhisha and Subramanian, (2005) studied in-vitro antifungal activity of *Pallavicina lyelli.* Diverse organic solvent extracts of *P. lyelli* indicated variable levels of activity against the test fungi. Spjut *et al* (1992) reported the first discovery of significant biological activity of moss *Polytrichum ohioense*.

Bodade *et al* (2008) screened mosses extracts to find out their antimicrobial activity against selected bacterial and fungal strains and noted promising effect on growth inhibition of test microorganisms. They also have observed that the ethanolic extract was more active than other fractioned extracts. They are of the opinion that antimicrobial activity may be due to secondary metabolites.

Antifungal activity of extracts of *Hyophila rosea* Williams, *Targionia hypophylla* L. and *Plagiochasma rupestre* (J.R. Forst. & G. Forst.) Steph were tested against fungal strains like *Alternaria alternata*, *Aspergillus niger, A. flavus*, *Phytophthora infestans*, *Trichoderma viride*, *Fusarium oxysporium, Penicillium chrysogenum P. expansum* and *Botrytis cinerea* and it was found that these plants were even more powerful antifungal agents than the synthetic fungicides at an average concentration of plant extract ranging from 2.5mg/ml to 20mg/ml (Alam, 2011; 2013).

Deora and Guhil. (2013) evaluated the antifungal potential and the phytochemical screening of *Bryum cellular*. They tested aqueous and ethanolic crude extracts against percentage inhibition of spore germination and hyphal length of test fungi *Curvularia lunata.* The results showed that all the extracts possess a significant antifungal potential but highest inhibition of percentage in spore germination was observed in ethanolic extract of *Bryum cellulare*.

On the other hand, our study showed that selected mosses samples in our study have antifungal activity against two selected fungi. While *S. cerevisiae* was sensitive against the five plant extracts, this strain was resistance against *T. tortuosa* extracts. However, acetone extract of *T. tortuosa* was only active against the *C. albicans*.

Our results revealed that the selected mosses might possess a novel antimicrobial agents. This study help in the discovery of new antibiotics that could serve as selective agents

against infectious diseases. Different organic solvents and aqueous extracts of mosses shows variable inhibitory activity against studied fungal strains in this study.

Table 12: Antifungal activity of methanolic extract against test organisms

Sr. No	Name of Mosses	Inhibition zone (mm)			
		F.oxysporum	*A. niger*	*T. viride*	*P. notatum*
1	*Funaria hygrometrica* Hedw	0.8 ± 0.1	0.9 ± 0.4	0.8 ± 0.2	1.0 ± 0.2
2	*Macromitrium sulcatum* Brid	0.6 ± 0.5	0.6 ± 0.2	0.8 ± 0.2	0.6 ± 0.1
3	*Brachymenium turgidum* Broth	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.0	0.4 ± 0.1
4	*Bryum coronatum* Schwaegr	0.6 ± 0.3	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.4
5	*Hyophila involuta* (Hook) Jaeg.	0.7 ± 0.4	0.6 ± 0.2	0.5 ± 0.2	0.4 ± 0.1

6	*Bryum ghatens* Broth. et Dix.	0.9 ± 0.5	0.7 ± 0.4	0.8 ± 0.4	0.7 ± 0.1
7	*Hypnum reflexum* F. E. Tripp.	0.9 ± 0.6	0.8 ± 0.2	0.9 ± 0.4	0.8 ± 0.1
8	*Fissidens crenulatus* Mitt.	0.7 ± 0.3	0.7 ± 0.1	0.9 ± 0.4	0.7 ± 0.2
9	*Trachypodiopsis blanda (*Mitt.) Fleisch.	0.6 ± 0.1	0.6 ± 0.0	0.8 ± 0.3	0.6 ± 0.2
10	*Steeriophyllum ancens (*Bosch et Lac.) Broth.	0.6 ± 0.1	0.7 ± 0.4	0.6 ± 0.2	1.3 ± 0.7
11	Antibiotics (Nastatin)	0.5 ± 0.1	0.6 ± 0.3	0.4 ± 0.1	0.5 ± 0.2

mm- Millimeter, *A-Aspergillus*, *P-Penicillium*, *F-Fusarium*, *T-Trichoderma*.

NA-No inhibition zone. Values are mean of three replications.

Fig 3: Antifungal activity of methanolic extract against test organisms

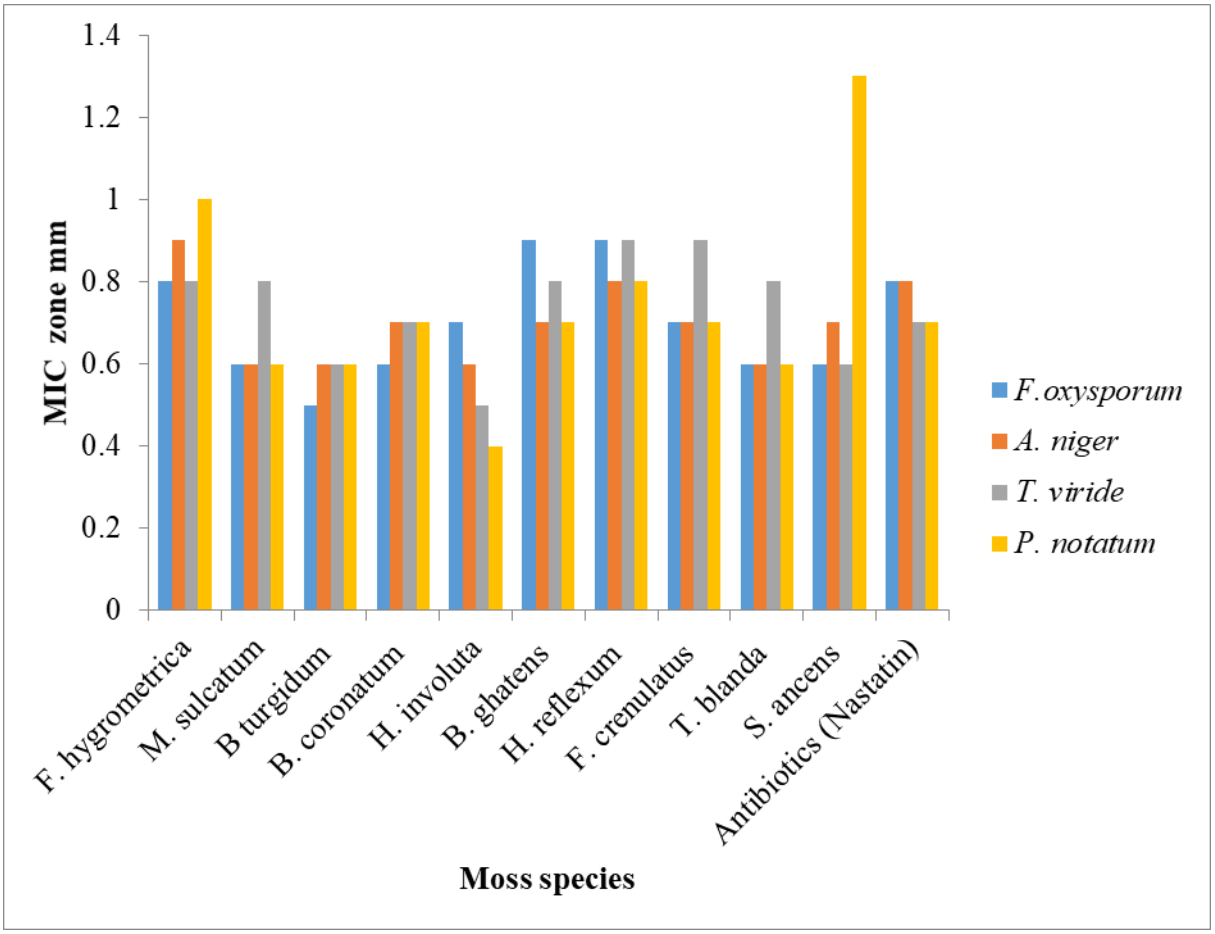

1.4
1.2
1
0.8
0.6
0.4
0.2
0
MIC zone mm
F. hygrometrica
M. sulcatum
B turgidum
B. coronatum
H. involuta
B. ghatens
H. reflexum
F. crenulatus
T. blanda
S. ancens
Antibiotics (Nastatin)
Moss species
F.oxysporum
A. niger
T. viride
P. notatum

Table 13: Antifungal activity of ethanolic extract against test organisms

Sr. No.	Name of Mosses	Inhibition zone (mm)			
		F.oxysporum	*A. niger*	*T. viride*	*P. notatum*
1	*Funaria hygrometrica* Hedw	0.6 ± 0.1	0.6 ± 0.5	0.6 ± 0.1	0.7 ± 0.3
2	*Macromitrium sulcatum* Brid	0.7 ± 0.3	0.8 ± 0.1	0.6 ± 0.0	0.7 ± 0.5
3	*Brachymenium turgidum* Broth	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.1
4	*Bryum coronatum* Schwaegr	0.6 ± 0.3	0.8 ± 0.4	0.7 ± 0.1	0.7 ± 0.1
5	*Hyophila involuta* (Hook) Jaeg.	0.9 ± 0.5	0.3 ± 0.1	0.6 ± 0.2	0.6 ± 0.1
6	*Bryum ghatens* Broth. et Dix.	0.9 ± 0.1	0.7 ± 0.3	0.8 ± 0.2	0.7 ± 0.5
7	*Hypnum reflexum* F. E. Tripp.	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.4	1.0 ± 0.4
8	*Fissidens crenulatus* Mitt.	0.5 ± 0.2	0.5 ± 0.0	0.6 ± 0.2	0.5 ± 0.2
9	*Trachypodiopsis blanda (*Mitt.) Fleisch.	0.6 ± 0.0	0.6 ± 0.3	0.6 ± 0.1	0.8 ± 0.1
10	*Steeriophyllum ancens* (Boschet Lac.) Broth.	0.6 ± 0.2	0.6 ± 0.0	0.5 ± 0.1	0.4 ± 0.1
11	Antibiotics (Nastatin)	0.5 ± 0.1	0.6 ± 0.4	0.4 ± 0.0	0.5 ± 0.3

mm- Millimeter, *A-Aspergillus*, *P-Penicillium*, *F-Fusarium*, *T-Trichoderma*.

NI-No inhibition zone. Values are mean of three replications.

Fig 4: Antifungal activity of ethanolic extract against test organisms.

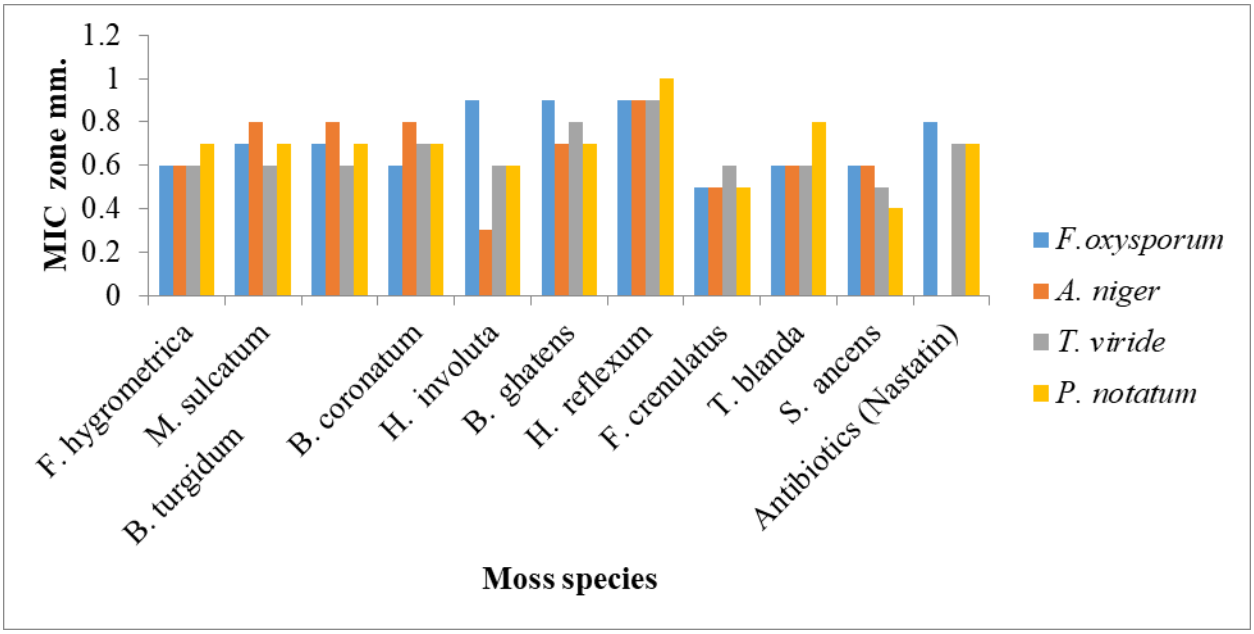


Table 14: Antifungal activity of petroleum ether extract against test organisms

Sr. No.	Name of Mosses	Inhibition zone (mm)			
		A. niger	*P. notatum*	*F.oxysporum*	*T. viride*
1	*Funaria hygrometrica* Hedw	0.6 ± 0.4	0.7 ± 0.2	0.4 ± 0.1	0.6 ± 0.1
2	*Macromitrium sulcatum* Brid	0.4 ± 0.0	0.6 ± 0.1	0.6 ± 0.2	0.4 ± 0.3
3	*Brachymenium turgidum* Broth	NI	NI	0.5 ± 0.1	0.3 ± 0.2
4	*Bryum coronatum* Schwaegr	NI	NI	NI	NI
5	*Hyophila involuta* (Hook) Jaeg.	0.3 ± 0.1	0.5 ± 0.2	0.6 ± 0.4	0.6 ± 0.0
6	*Bryum ghatens* Broth. et Dix.	0.5 ± 0.1	NI	0.1± 0.0	NI
7	*Hypnum reflexum* F. E. Tripp.	NI	NI	NI	NI
8	*Fissidens crenulatus* Mitt.	0.2 ± 0.1	NI	NI	NI
9	*Trachypodiopsis blanda (*Mitt.) Fleisch.	NI	NI	NI	NI
10	*Steeriophyllum ancens* (Bosch et Lac.) Broth.	NI	0.2 ± 0.0	0.2 ± 0.1	NI
11	Antibiotics (Nastatin)	0.5 ± 0.2	0.6 ± 0.1	0.4 ± 0.3	0.5 ± 0.1

mm- Millimeter, *A-Aspergillus, P-Penicillium, F-Fusarium, T-Trichoderma.*

NI-No inhibition zone. Values are mean of three replications.

Fig 5: Antifungal activity of petroleum ether extract against test organisms

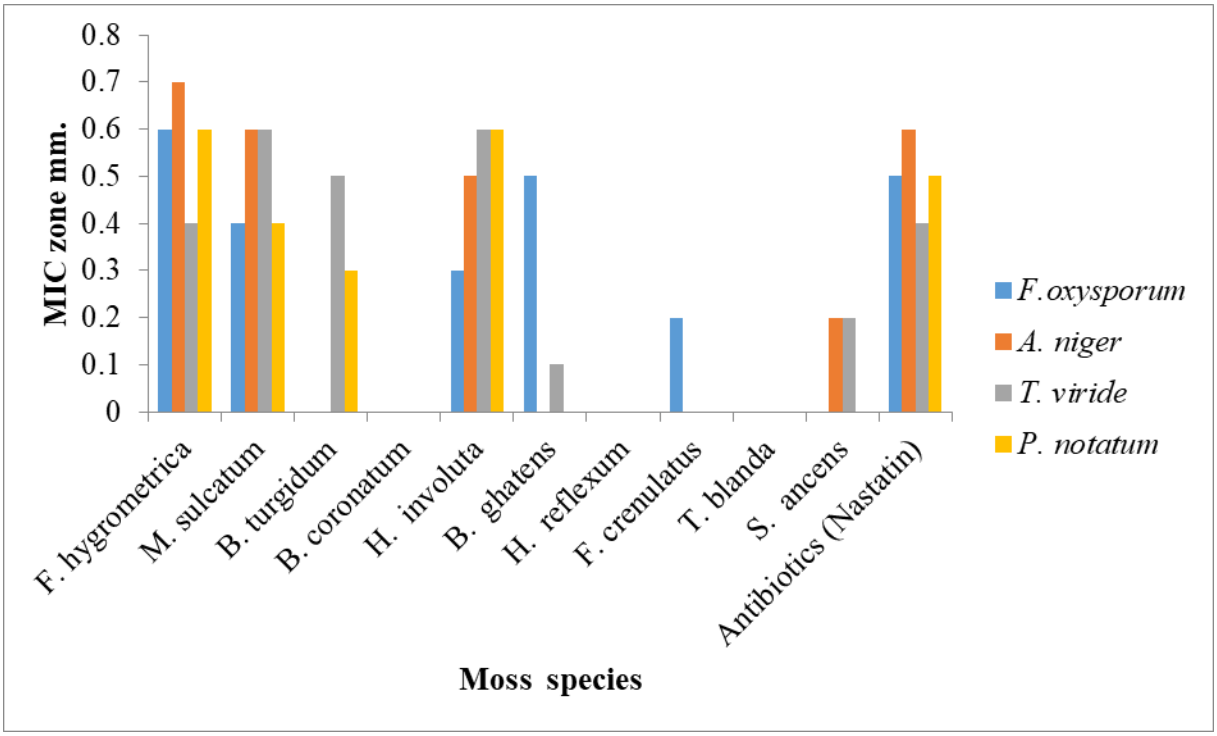


Table 15: Antifungal activity of aqueous extract against test organism

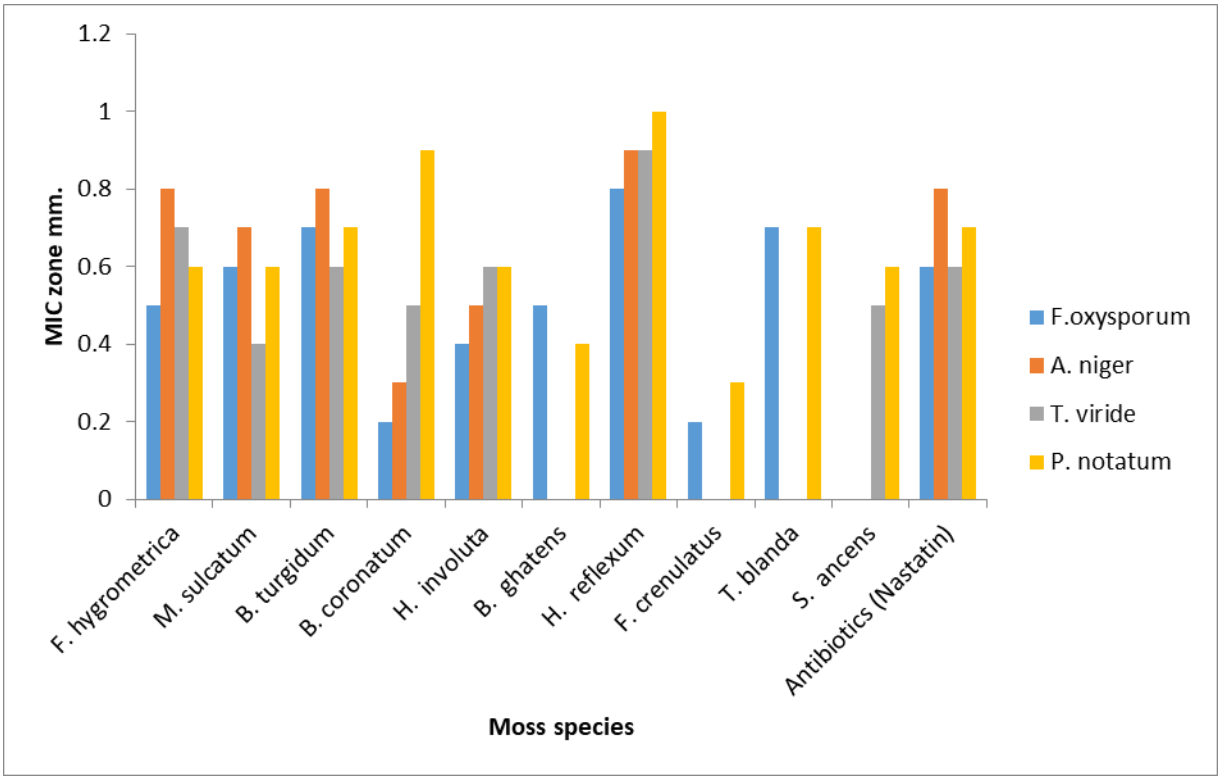
Sr.	Name of Mosses	Inhibition zone (mm)

No.		*A. niger*	*P. notatum*	*F.oxysporum*	T. *viride*
1	*Funaria hygrometrica* Hedw	0.5 ± 0.1	0.8 ± 0.5	0.7 ± 0.2	0.6 ± 0.1
2	*Macromitrium sulcatum* Brid	0. 6 ± 0.4	0.7 ± 0.1	0.4 ± 0.1	0.6 ± 0.2
3	*Brachymenium turgidum* Broth	0.7 ± 0.5	0.8 ± 0.1	0.6 ± 0.1	0.7± 0.5
4	*Bryum coronatum* Schwaegr	0.2 ± 0.0	0.3 ± 0.2	0.5 ± 0.4	0.9 ± 0.6
5	*Hyophila involuta* (Hook) Jaeg.	0.4 ± 0.1	0.5 ± 0.2	0.6 ± 0.3	0.6 ± 0.4
6	*Bryum ghatens* Broth. et Dix.	0.5 ± 0.1	NI	NI	0.4 ± 0.1
7	*Hypnum reflexum* F. E. Tripp.	0.8 ± 0.2	0.9 ± 0.1	0.9 ± 0.5	1.0 ± 0.4
8	*Fissidens crenulatus* Mitt.	0.2 ± 0.1	NI	NI	0.3 ± 0.1
9	*Trachypodiopsis blanda (*Mitt.) Fleisch.	0.7 ± 0.3	NI	NI	0.7± 0.1
10	*Steeriophyllum ancens (*Bosch et Lac.) Broth.	NI	NI	0.5 ± 0.0	0.6 ± 0.2
11	Antibiotics (Nastatin)	0.5 ± 0.1	0.6 ± 0.4	0.4 ± 0.2	0.5 ± 0.1

mm -Millimeter, *A-Aspergillus, P-Penicillium, F-Fusarium, T-Trichoderma.*

NI-No inhibition zone. Values are mean of three replications.

Fig 6: Antifungal activity of aqueous extract against test organism



B. Antibacterial activity:

Antibacterial activity of methanolic extract shows maximum zone 1.3mm against *Bryum coronatum* for *Pseudomonas aeruginosa* (Table 14) and minimum 0.1 mm for *Steeriophyllum*

anceps against *Bacillus substilis*, remaining mosses shows minimum zone from 0.3 mm to 1.2 mm against all tested bacteria. Ethanolic extracts shows maximum zone of inhibition 1.0 mm (Table 15) against *Bryum coronatum* and *Macromitrium sulcatum* against *E coli*. *Hypnum reflexum* shows minimum zone of inhibition 0.3 mm against *P aeruginosa.*

Petroleum ether extracts showed maximum antibacterial activity 1.2mm for *Macromitrium sulcatum* against *E. coli* and 1.0 for *Staphylococcus aureus*. Minimum zone 0.4 mm noted for *Bryum ghatens, Hypnum reflexum* and *Hypnum reflexum. Bryum coronatum* does not shows inhibition zone against *Pseudomonas aeruginosa, E coli* and *B substilis*.

Aqueous extract of *Trachypodiopsis blanda* shows 1.2 mm inhibition zone (Table 17) against *P. aeruginosa* and Minimum zone 0.1mm for *Bryum ghatens* and *Hypnum reflexum* against *S. aureus.* No inhibition zone occurs for *Brachymenium turgidum*, *Trachypodiopsis blanda* and *Steeriophyllum anceps* against *S. aureus, P. aeruginosa and Trichoderma viride.* In this study the methanolic extracts from mosses demonstrated a poor effect against the selected bacteria species in general while ethanol, petroleum ether and water extracts had a higher potency.

Interestingly, all the mosses in the present study showed good antimicrobial potential against the tested organisms which may again comprise the synergistic effect of several other compounds in addition to phenolics and flavonoids. The results of the agar diffusion method in the present study revealed that of all the mosses species investigated, *Bryum coronatum* and *Macromitrium sulcatum* had stronger potential activity against the bacterial strains tested.

In the present study, ethanol extract showed a broader range of activity and thus exhibited good antibacterial action against most of the bacteria. This may be due to extraction of specific antibacterial compounds in the ethanolic extracts. Krishnan *et al.* (2012) also reported good antimicrobial activity of methanolic and water fractions of *Targionia hypophylla* and *Bryum sp.*

The difference in the antimicrobial activity may be due to potential differences in the strains of bacteria and used different extrahents and experimental procedures. The different antimicrobial activity of different moss species may also be attributed to the presence of a number of antimicrobial substances with different spectra of action and intensity in different

moss extracts. They reported that the activity may be due to the presence of various secondary metabolites (Basile *et al.,* 1999).

Ethanol extracts, compared to the methanol extracts, exhibited higher activity. This may be due to different abilities of different solvents to extract different active compounds depending on their solubility or polarity in the solvent (Marjorie (1999). Ethanol extract in this study might have had a higher solubility for more number or more concentration of active compounds and therefore exhibited higher activity.

The antibacterial activity of some mosses against certain Gram-negative bacteria has been shown in other studies like, *Leptodictyum riparium* extract is able to inhibit growth of Gram-negative bacteria more than Gram-positive; its extract is also mostly active against conventional antibiotic resistant species like *Pseudomonas aeruginosa* (Castaldo-Cobianchi *et al*., 1988). This is of significant importance since conventional antibiotics are usually more active against Gram-positive bacteria.

Rodriguez *et al*., (1996) studied antibacterial activity of ethanolic extracts of two mosses viz: *Hypnum amabile* and *Sphagnum magellanicum* and two liverworts viz: *Metzgeria decipiens* and *Trichocolea tomentos* against *Bacillus subtalis, Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli* of commercial fruit juices and compared with Ampicillin and Clindamycin. Ethanolic extracts of all bryophytes were active against at least two of the tested bacteria with different magnitudes of inhibition. Ahmed *et al,* (1998) reported that the phytochemical compounds are responsible for the antimicrobial activity. As already mentioned, main interest in bryophytes chemical research is secondary metabolites with biological activity, extrahents mainly used in order to extract are such substances are methanol, ethanol, water and chloroform (Basile *et al.,* 1999; Sing *et al*., 2006; Saboljevic *et al*., 2010; Fu *et al.,* 2012).

Lhan *et al.,* (2006) studied antimicrobial activity of *Palustriella commutate* (Hedw.) Ochyra using the disc diffusion method against one yeast, eight moulds and eleven bacteria. Extracts showed remarkable activity against gram-negative and gram-positive bacteria. Sabovljevic *et al.,* (2006) studied ethanol extracts of *Bryum argenatum* for antimicrobial activity by micro dilution method. Extract was tested against four bacterial strains viz: *Candida albicans Micrococcus luteus, Bacillus subtalis, E coli,* and *Staphylococcus aureus* and four fungal strains

viz: *Aspergillus niger, Penicillium ochrochloron,* and *Trichophyton mentagrophyes*. Ethanol extracts have been proved to be active against all tested bacteria and fungi.

Atricum selwynii and *Sphagnum pulustre* have also revealed activity against a variety of Gram-positive and Gram-negative bacteria. *Hylocomium splendens* has shown antibiotic activity against 9 Gram-positive bacteria (Kang *et al*, 2007). Bodade *et al.* (2008) stated that ethanolic, acetone, and chloroform extracts of Bryophytes were found to be more effective than methanolic extract.

Studies carried out by Singh *et al*. (2011) also reveal that the bryophytes, namely, *Plagiochasma appendiculatum, Conocephalum coricum, Mnium marginatum,* and *Bryum argenteum* have antibacterial activity and their findings support the use of bryophytes in traditional medicine for treating burn infections. Similar observations have been made by Nikolejeva *et al.* (2012) in different bryophytes. They have found that 73% ethanolic extracts exhibited antibacterial activity and it was also higher as compared with aqueous extracts.

Extract of the mosses like *Funaria hygrometrica* Hedw, *Polytrichum juniperinum* Hedw. *Hypnum cupressiforme* Hedw*, H. imponens* Hedw, and *Tortella tortuosa* (Hedw.) Limpr were tested against six bacterial and three fungal strains to find out antibacterial and antifungal activities. Some of the extracts indicated activity on both Gram-positive and Gram-negative bacteria. Extract of *T. tortuosa* possessed the highest antibacterial activity against *Pseudomonas aeruginosa* (Savaroglu *et al*. 2011 a).

Deora and Rathore. (2013) reported antibacterial effect of *Plagiochasma articulatum* and *Fissidens bryoides* on some bacterial strains such as *Agrobacterium tumifaciens, Streptomyces scabies* and *Xanthmonas citri. Plagiochasma articulatum* extract was more potent than *Fissidens bryoides* against all tested bacterial strains. The antimicrobial activity carried out by Oyesiku and Caleb (2015) in three mosses also reveal that ethanol extract was found to be more active than the other two and are of the opinion that these extracts show antibiotic activity. This suggests that specific antibacterial compounds isolated by ethanol are more effective against specific bacteria.

In this study, four different solvent extracts (methanol, ethanol, petroleum ether and water) were used for each moss species. Different organic solvents and aqueous extracts of

mosses shows variable inhibitory activity against studied bacterial strains in this study. Mosses from Western Ghats of Maharashtra have reported to prevent the growth of microorganisms such as bacteria and fungi.

Our recent report on antimicrobial screening gives variable activities at different localities. The present study reports that there is varying level of activity in the test species. This suggests that the extracts in four solvents have a broad spectrum activity, hence there is a need to investigate further for their use as for antibacterial agents, which could help in new drug development.

The antimicrobial activities of mosses extracts has not been previously reported from studied localities of Western Ghats of Maharashtra. Our results have evaluated as first time report on antimicrobial properties of mosses extracts from Western Ghats of Maharashtra. With connection of these studies our results indicates that the antimicrobial activities of mosses. The antimicrobial activity of extracts is due to presence of secondary metabolites or bioactive compounds. The screening of mosses for antibacterial and antifungal activities are due to potentially rich source of antimicrobial agents. This study indicates that the mosses possess a novel bioactive compounds which has an inhibitory effect against the tested fungal and bacterial strains. Our results indicates that varying levels of antifungal and antibacterial activity against tested strains. It is hoped that, this piece of work will unravel many complicated problems pertaining to antibiotic properties of mosses and will also give a guideline to future work.

Table 16: Antibacterial activity of methanolic extract against test organisms

Sr. No.	**Name of Mosses**	**Inhibition zone (mm)**			
		S. aureus	*P. aeruginosa*	*E.coil*	*B. substilis*
1	*Funaria hygrometrica* Hedw	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.4	0.6 ± 0.1
2	*Macromitrium sulcatum* Brid	1.0 ± 0.4	0.9 ± 0.3	1.0 ± 0.1	0.9 ± 0.1
3	*Brachymenium turgidum* Broth	0.8 ± 0.1	0.9 ± 0.2	1.2 ± 0.7	0.7 ± 0.4

4	*Bryum coronatum* Schwaegr	0.9 ± 0.4	1.3 ± 0.5	0.9 ± 0.3	0.6 ± 0.2
5	*Hyophila involuta* (Hook) Jaeg.	0.3 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.3 ± 0.1
6	*Bryum ghatens* Broth. et Dix.	0.8 ± 0.3	1.2 ± 0.5	0.7 ± 0.2	0.6 ± 0.1
7	*Hypnum reflexum* F. E. Tripp.	0.6 ± 0.1	0.7 ± 0.3	0.8 ± 0.4	0.9 ± 0.5
8	*Fissidens crenulatus* Mitt.	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
9	*Trachypodiopsis blanda (*Mitt.) Fleisch.	0.7 ± 0.1	0.8 ± 0.3	0.6 ± 0.1	0.8 ± 0.2
10	*Steeriophyllum ancens (*Bosch et Lac.) Broth.	0.7 ± 0.2	0.6 ± 03	0.4 ± 01	0.1 ± 00
11	Antibiotics (Ampicillin)	0.6 ± 0.1	0.8 ± 0.2	0.8 ± 0.3	0.6 ± 0.1

mm- Millimeter, *S- Staphylococcus, P- Pseudomonas, E- Escherichia, B- Bacillus*

NI-No inhibition zone. Values are mean of three replications.

Fig 7: Antibacterial activity of methanolic extract against test organisms

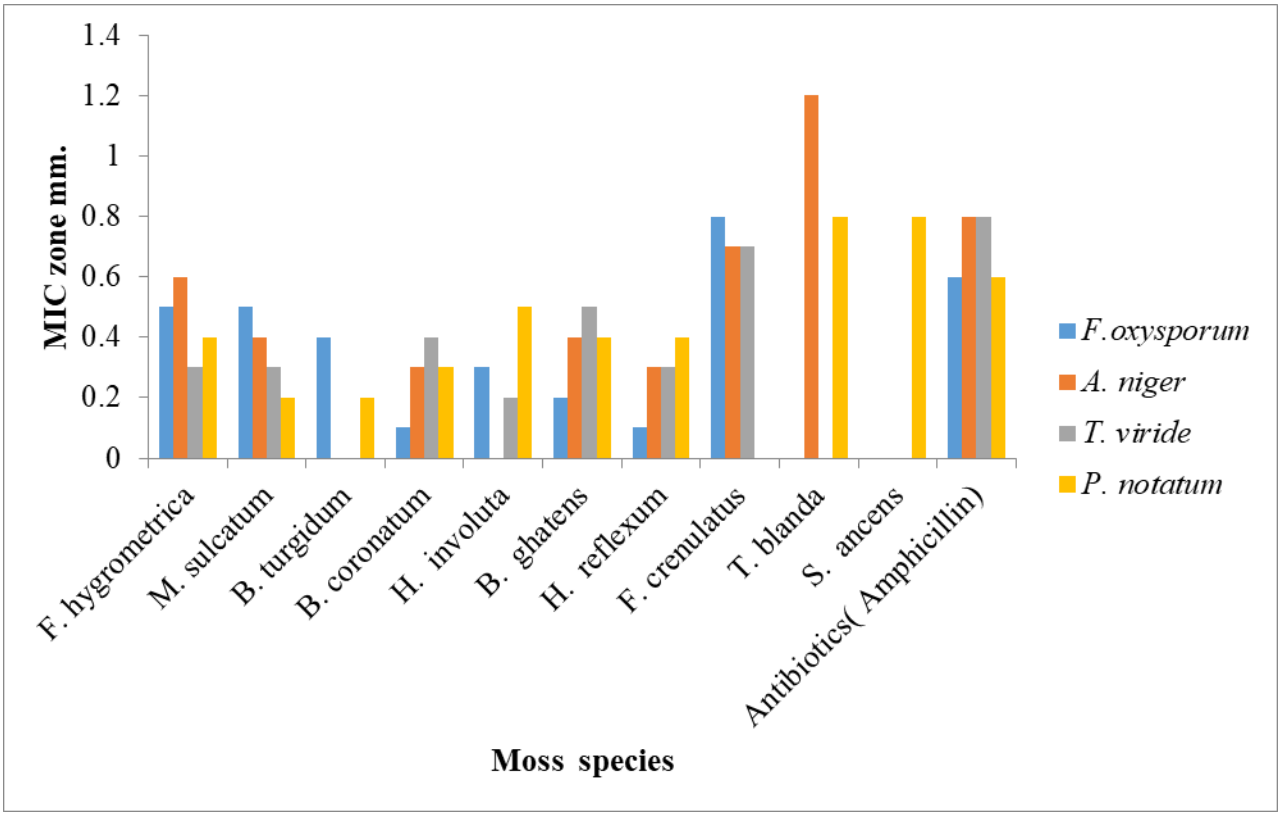


Table 17: Antibacterial activity of ethanolic extract against test organisms

Sr. No.	Name of Mosses	Inhibition zone (mm)			
		S. aureus	*P. aeruginosa*	*E.coil*	*B. substilis*
1	*Funaria hygrometrica* Hedw	0.8 ± 0.3	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.2
2	*Macromitrium sulcatum* Brid	0.5 ± 0.1	0.6 ± 0.1	**1.0** ± 0.3	0.9 ± 0.3
3	*Brachymenium turgidum* Broth	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.6 ± 0.2
4	*Bryum coronatum* Schwaegr	0.8 ± 0.2	0.9 ± 0.2	**1.0** ± 0.2	0.7 ± 0.2
5	*Hyophila involuta* (Hook) Jaeg.	0.6 ± 0.2	0.9 ± 0.2	**1.0** ± 0.2	0.7 ± 0.2
6	*Bryum ghatens* Broth. et Dix.	0.7 ± 0.2	0.6 ± 0.2	0.9 ± 0.2	0.7 ± 0.2
7	*Hypnum reflexum* F. E. Tripp.	0.6 ± 0.2	**0.3** ± 0.2	0.6 ± 0.2	0.7 ± 0.2
8	*Fissidens crenulatus* Mitt.	0.7 ± 0.2	NI	NI	0.5 ± 0.2
9	*Trachypodiopsis blanda (*Mitt.) Fleisch.	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
10	*Steeriophyllum ancens (*Bosch et Lac.) Broth.	0.9 ± 0.2	0.5 ± 0.2	0.8 ± 0.2	0.7 ± 0.2
11	Antibiotics(Ampicillin)	0.6 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.6 ± 0.2

mm- Millimeter, *S- Staphylococcus, P- Pseudomonas, E- Escherichia, B- Bacillus*

NI-No inhibition zone. Values are mean of three replications.

Fig 8: Antibacterial activity of ethanolic extract against test organisms

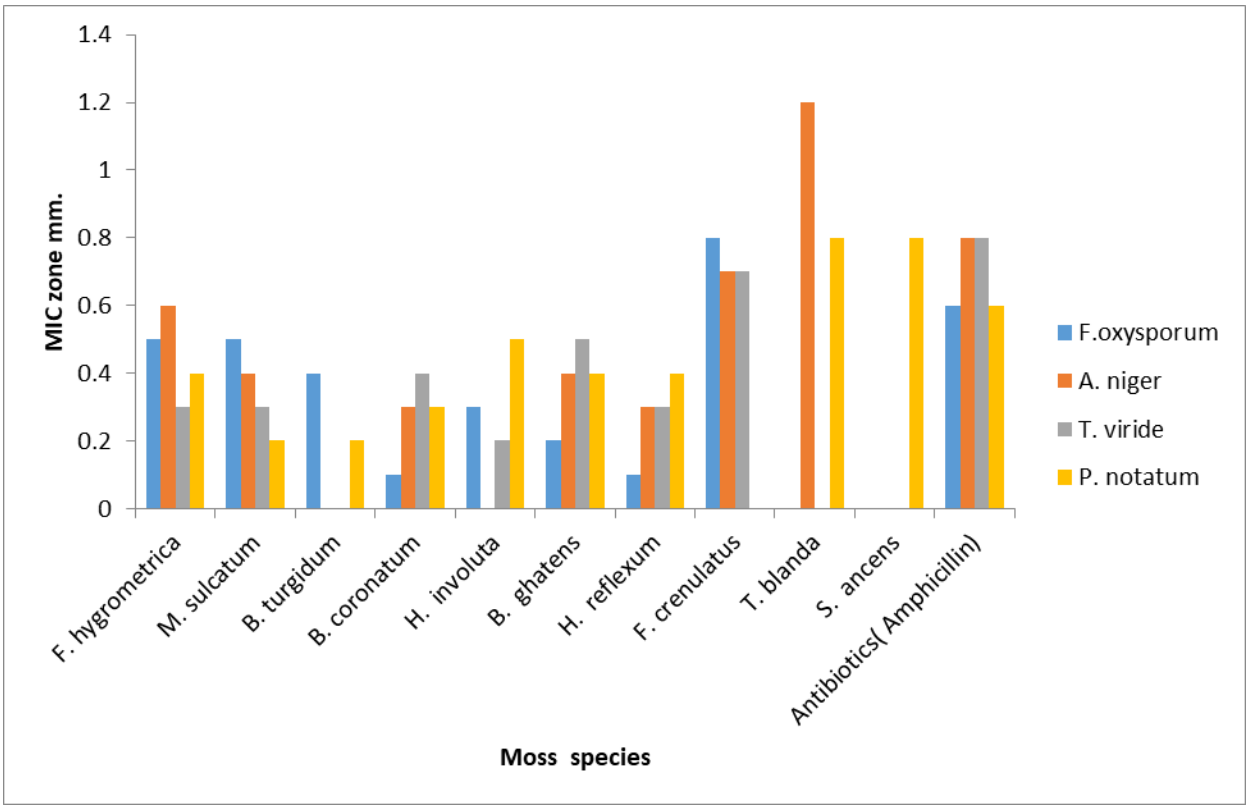

1.4
1.2
1
0.8
0.6
0.4
0.2
0
MIC zone mm.
F. hygrometrica
M. sulcatum
B. turgidum
B. coronatum
H. involuta
B. ghatens
H. reflexum
F. crenulatus
T. blanda
S. ancens
Antibiotics(Amphicillin)
Moss species
F.oxysporum
A. niger
T. viride
P. notatum

Table 18: Antibacterial activity of petroleum ether extract against test organisms

Sr No	Name of Mosses	Inhibition zone (mm)			
		S. aureus	*P. aeruginosa*	*E.coil*	*B. substilis*
1	*Funaria hygrometrica* Hedw	NI	NI	NI	NI
2	*Macromitrium sulcatum* Brid	**0.4** ± 0.1	0.5 ± o.1	**1.2** ± 0.5	0.8 ± 0.2
3	*Brachymenium turgidum* Broth	0.5 ± 0.2	NI	NI	NI
4	*Bryum coronatum* Schwaegr	0.8 ± 0.3	0.7 ± 0.1	**0.4** ± 0.0	0.6 ± 0.1
5	*Hyophila involuta* (Hook) Jaeg.	0.6 ± 0.2	0.5 ± 0.1	NI	**0.4** ± 0.1
6	*Bryum ghatens* Broth. et Dix.	NI	NI	0.7 ± 0.4	NI
7	*Hypnum reflexum* F. E. Tripp.	NI	NI	NI	NI
8	*Fissidens crenulatus* Mitt.	**1.0** ± 0.4	NI	NI	0.9 ± 0.2
9	*Trachypodiopsis blanda (*Mitt.) Fleisch.	0.9 ± 0.3	NI	NI	0.9 ± 0.1
10	*Steeriophyllum ancens (*Bosch et Lac.) Broth.	NI	NI	NI	NI
11	Antibiotics(Ampicillin)	0.6 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	0.6 ± 0.1

mm- Millimeter, *S- Staphylococcus, P- Pseudomonas, E- Escherichia, B- Bacillus*

NI-No inhibition zone. Values are mean of three replications.

Fig 9: Antibacterial activity of Petroleum ether extract against test organisms

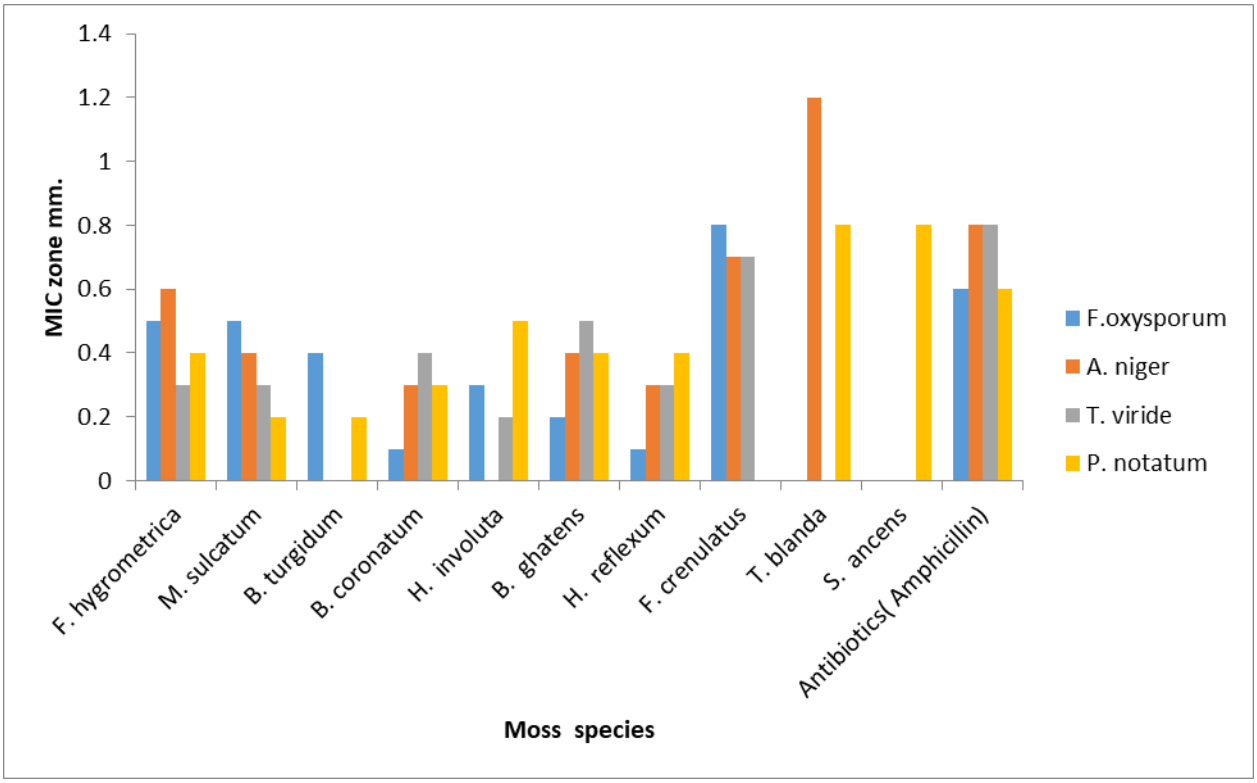

1.4
1.2
1
0.8
0.6
0.4
0.2
0
MIC zone mm.
F. hygrometrica
M. sulcatum
B. turgidum
B. coronatum
H. involuta
B. ghatens
H. reflexum
F. crenulatus
T. blanda
S. ancens
Antibiotics(Amphicillin)
Moss species
F.oxysporum
A. niger
T. viride
P. notatum

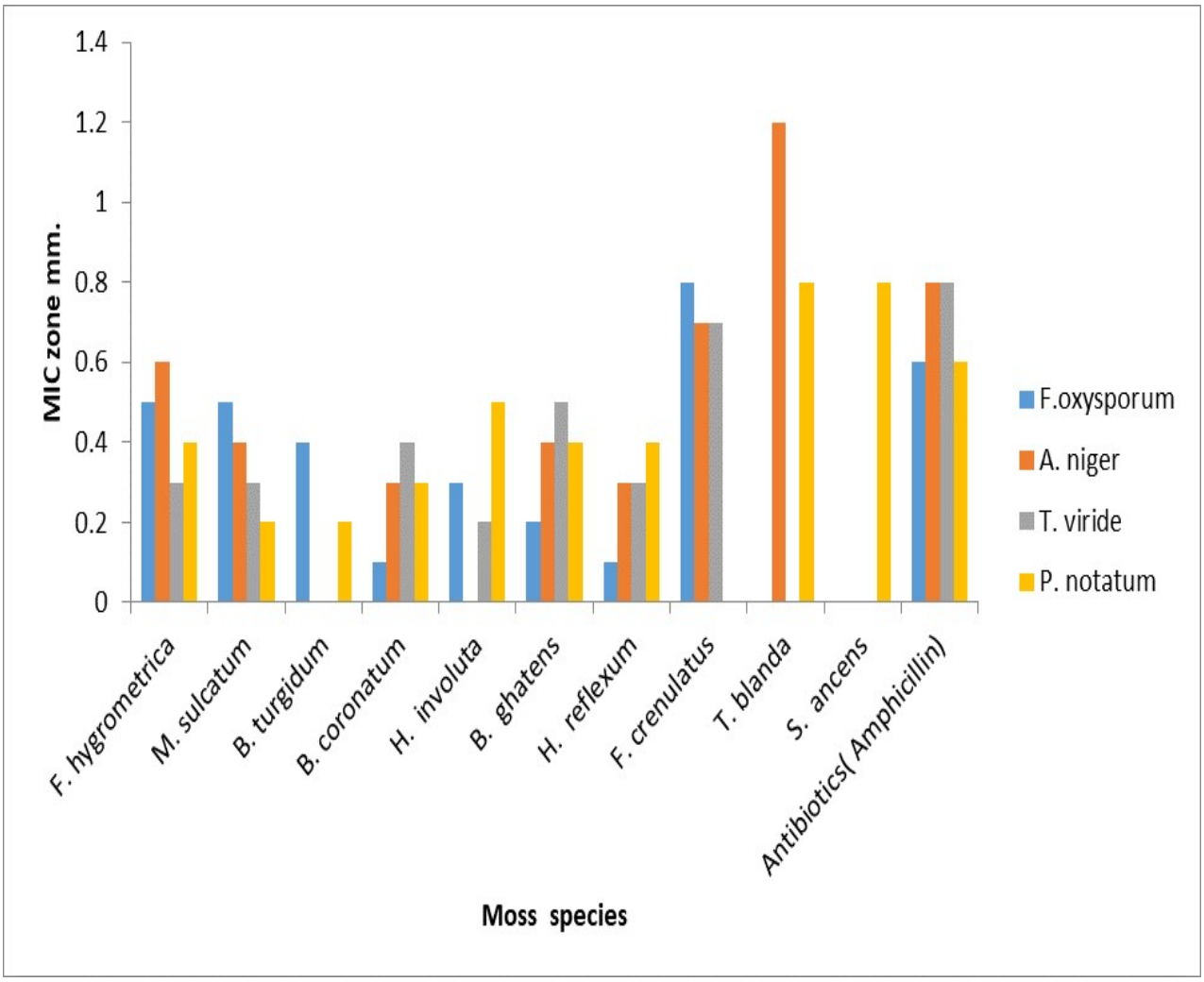
Table 19: Antibacterial activity of aqueous extract against test organisms

Sr No	Name of Mosses	Inhibition zone (mm)			
		S. aureus	*P. aeruginosa*	*E.coil*	*B. substilis*
1	*Funaria hygrometrica* Hedw	0.5 ± 0.2	0.6 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
2	*Macromitrium sulcatum* Brid	0.5 ± 0.3	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
3	*Brachymenium turgidum* Broth	0.4 ± 0.1	NI	NI	0.2 ± 0.1
4	*Bryum coronatum* Schwaegr	**0.1** ± 0.0	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1
5	*Hyophila involuta* (Hook) Jaeg.	0.3 ± 0.1	NI	0.2 ± 0.0	0.5 ± 0.2
6	*Bryum ghatens* Broth. et Dix.	0.2 ± 0.0	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1
7	*Hypnum reflexum* F. E. Tripp.	**0.1** ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
8	*Fissidens crenulatus* Mitt.	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	0.2 ± 0.0
9	*Trachypodiopsis blanda (*Mitt.) Fleisch.	NI	**1.2** ± 0.5	NI	0.8 ± 0.2
10	*Steeriophyllum ancens (*Bosch et Lac.) Broth.	NI	NI	NI	0.8 ± 0.3
11	Antibiotics (Ampicillin)	0.6 ± 0.1	0.8 ± 0.2	0.8 ± 0.4	0.6 ± 0.2

mm- Millimeter, *S- Staphylococcus, P- Pseudomonas, E- Escherichia, B- Bacillus*

NI-No inhibition zone. Values are mean of three replications.

Fig 10: Antibacterial activity of aqueous extract against test organisms



4.5 Isolation of metabolites:

Preliminary phytochemical analysis of extracts of mosses shown that presence of alkaloids, glycosides phenols, saponins, tannins and flavonoids. The phytochemical analysis of the mosses are important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new medicines for treatment of various diseases. The earlier phytochemical analysis and present study showed almost the similar results due to the presence of the phytochemical constituents. It may be helpful for further studies and investigations in the fields of phytochemistry, pharmacology and drug industry.

4.5.1 Qualitative phytochemical analysis

The qualitative methods used to identify the presence of secondary metabolites like alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and glycosides. Alkaloids, tannins, terpenoids, glycosides and saponins are completely absent. Flavonoids, phenols and steroids are detected in studied species. (Table 20). The antimicrobial activity in bryophytes has been attributed to the presence of flavonoids, steroids, terpenoids, triterpenoids, mono-, di- sesqui terpenoids, unsaturated lipids, fatty acids, esters, phenolics and other polyphenolic compounds (Tutschek and Rudolph, 1971).

Many botanists and microbiologists have documented the presence of antibiotic substances and biologically active compounds in bryophytes such as glycosides, terpenoids, phenols, and fatty acids (Banerjee and Sen, 1979, Glime and Saxena 1991, Zhu *et al*., 2006 and Sabovljevic *et al.,* 2009). They contain several potential compounds including polysaccharides, amino acids, sugars, alcohols and phenolic compounds (Pant and Tiwari 1990). The constituents that have been isolated from *Hypnum cupressiforme* are hypnogenols, biflavonoids and dihydroflavonols. All the flavonoids showed good antimicrobial activity against the tested bacteria and the highest activity that of saponarine. Some of these flavonoids were shown to have marked antibacterial effects (Dulger *et al.,* 2005).

Biflavonoids in mosses have been reported as potential agents against certain pathogenic microorganisms (Lopez-Saez, 1996). Basilea *et al.,* (1999) isolated and identified seven pure flavonoids from studied mosses. These flavonoids were the flavones apigenin, lucenin-2, apigenin7-O-triglycoside, luteolin-7-O-neohesperidoside, saponarine, vitexin and the bifavonoid bartramiafavone. Out of these favonoids same shown antibacterial effects against *Enterobacter aerogenes, E. cloaceae,* and *Pseudomonas aeruginosa.* Batish *et al*. (1997), Ahmed *et al.* (1998) stated that the antimicrobial activity may be due to presence of various secondary

metabolites. Xu and Lee, (1999) reported the antibacterial activity of flavonoids .Among the flavonoids examined, four flavonols viz: myricetin, kaempferol, datiscetin and quercetin and two flavones viz: flavone and luteolin showed inhibitory activity against *Staphylococcus aureus* which is methicillin-resistant bacterium. Myricetin was found to inhibit the growth of the vancomycin-resistant enterococci and also against multidrug-resistant bacterium *Burkholderia cepacia.*

Since flavonoids are known to be produced by plants in response to microbial infection, it is not amazing that they have been found to be active antimicrobial constituents against microorganisms. Some lipophilic flavonoids may also interrupt microbial membranes (Basile *et al*., 1999; Cowan, 1999., Jockovic *et al*., 2008). Terpenoids, phenolic and volatile constituents have also been investigated in some bryophytes. Many of the terpenoids were described and isolated mainly from liverworts (Saritas, 2001).

Qualitative phytochemical analysis of a moss *Bryum argenatum* extract was done to detect the presence or absence of certain bioactive compounds by the methods of Trease and Evans (2002). They have been found in vitro to be effective antimicrobial substances against a variety of micro-organisms. Extract of *Funaria hygrometrica* and *Hypnum cupressiforme* showed less antifungal activity against *G. candidum*. It has been reported in previous studies that *Hypnum cupressiforme* had antifungal activity and that it contained biflavonoids, hydroxiflavonoids, polycyclic aromatic hydrocarbon and hypnogenols (Dulger *et al.,* 2005)

It is interesting that liverworts and moss species are significant source of tetraterpenoids and carotenoids. These activities may be due to the existence of carotenoids like α- and β-carotene, neo-, viola-, lutein, crypto-and xanthine (Sabovljevic 2008). Terpenoids represent one more class of secondary metabolites which advantage the producing organisms with increased pathogen resistance (Xie and Lou, 2009). Asakawa (2012) find out that some terpenoids isolated from liverworts show specific scents, pungent and bitterness taste, allergenic effect, cytotoxicity, anti-HIV inhibitory, antimicrobial, insect antifeedant mortality, dermatitis, and nematocidal activity.

Deora (2015) has observed that selected bryophytes showed the presence of terpenoids, flavonoids, steroids, and glycosides were present whereas, whereas alkaloids, saponins and anthroquinons were not present and have further stated that these chemical compounds could be potent antimicrobial agents to treat plant diseases.

The data we obtained for *Bryum coronatum* seems to support the presence of these phytochemicals (Table). In this study, one or more of the antifungal activities observed in the extracts from *F. hygrometrica, H. cupressiforme* and *T. tortuosa* could be said to be active terpenoids. The results of the phytochemical screening reveals that the phytochemical compounds may be responsible for antimicrobial activity of the mosses and they are alkaloids, flavonoids, resins, steroids and tannins.

Table 20: Phytochemical analysis of secondary metabolites.

Sr. No	Name of secondary metabolites.	Test Present or Absent

1	Alkaloids	-
2	Flavonoids	+
3	Phenols	+
4	Tannins	
5	Steroids	+
6	Terpenoids	-
7	Saponins	-
8	Glycosides	-

Present: +, Absent: -

Table 21 Qualitative screening of phytochemical constituents (According to the procedure of Harborne, 1998).

No	Experiments/ Test	Observation and Inference
1	Test for alkaloids 1 ml of test solution shaken with 2N HCl. Aqueous layer formed, decanted and to which one or two drops of Mayer's reagent added.	White turbidity or precipitate develops. Presence of alkaloids

2	Test for steroids 1 ml of test solution + 3 – 4 drops of chloroform and few drops of acetic acid, acetic anhydride and 2 drops of Con. H_2SO_4 and heated gently.	Blue or green colour develops. Presence of Steroids.
3	Test for tannins 1 ml of test solution + H_2O + lead acetate	White precipitate develops Presence of tannins
4	Test for saponins 1 ml of test solution + 1 ml of distilled water and mixed well	Foamy lather develops Presence of Saponins
5	Test for flavonoids In a test tube few drops of 1% NH_3 solution is added to the 1 ml test solution	Appearance of yellow colour.
6	Test for terpenoids 5 ml of plant sample is mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of con H_2SO_4 is carefully added to the mixture to form a layer	An interface with a reddish brown Coloration is formed.
7	Test for cardiac glycosides 5 ml of the plant sample is mixed with 2 ml of glacial acetic acid containing 1 drop of $FeCl_3$. The above mixture is carefully added to the 1 ml con H_2SO_4 so that the con H_2SO_4 is underneath the mixture.	A brown ring will appear Presence of cardiac glycosides
8	Test for phenolic compounds Alcoholic solution of test solution (1 ml) + one drop of ferric chloride	Intense colour develops Presence of Phenolic groups.

Table 22: Analysis of secondary metabolites by HPLC

PDA Ch 1) 254 nm

Peak	Ret. Time	Area	Height	Conc.
1	1.219	275986	58337	35.877
2	1.763	16805	869	2.185
3	2.570	106175	8870	13.802

4	3.245	283216	32137	36.817
5	3.423	42788	6129	5.562
6	3.578	21135	2186	2.747
7	3.794	17523	2901	2.278
8	5.141	1663	106	0.216
9	5.536	1835	139	0.239
10	5.752	2125	222	0.276
Total		769251	111895	

Table 23: Analysis of secondary metabolites by HPLC

PDA Ch 2) 280 nm

Peak	Ret. Time	Area	Height	Conc.
1	1.219	189479	39907	46.232
2	1.764	9908	524	2.417
3	2.570	61558	6478	15.020
4	3.256	92992	17839	22.690

5	3.426	30841	4724	7.525
6	3.578	9787	1159	2.388
7	3.793	15282	2439	3.729
Total		409847	73070	

Table 24 : Analysis of secondary metabolites by HPLCPDA Ch 3) 330 nm

Peak	Ret. Time	Area	Height	Conc.
1	1.220	120392	26130	46.232
2	2.519	14521	1721	2.417
3	3.256	30215	7395	15.020
Total		165127	35247	

Fig: 11. HPLC spectrum of 254nm

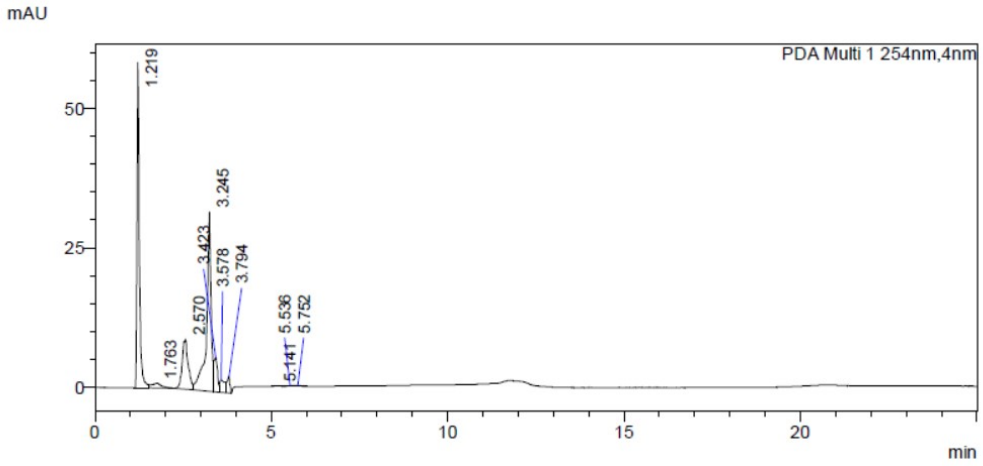


Fig: 12. HPLC spectrum of 280nm

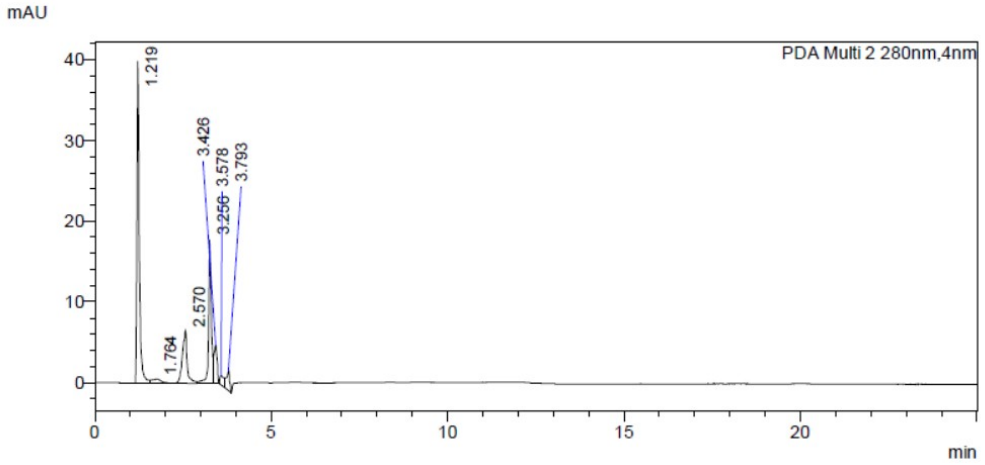
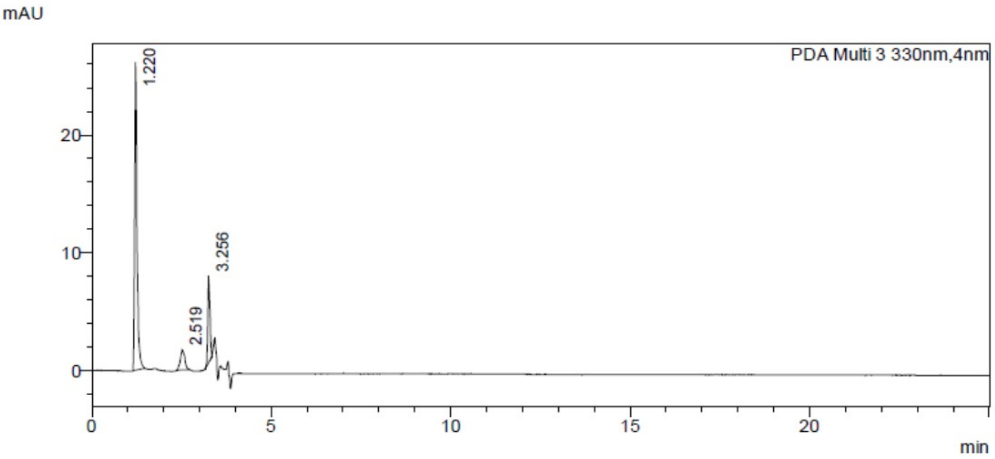


Fig: 13. HPLC spectrum of 330 nm



4.5.2 High Pressure Liquid Chromatography (HPLC)

At 254 nm we got peak at 3.24 minute retention time, concentration was 36.81 %, whereas at 280 nm retention time was1.2 minutes for component showing 46.23% and 3.2 minutes for compound 22.69%. Lastly at 330 nm we got retention time 1.2 minutes showing 46% concentration and at3.2 minutes 15.02%. Retention time of quercetin is 3.2 minutes and other components such as thymol, piperine and ferulic acid found at 30 minutes run time.

It can be concluded from the result that HPLC is a versatile Chromatographic technique for the estimation of drug products. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules.

4.5. 3 Gas Chromatography and mass spectroscopy

To trace the possible chemical compounds, Gas Chromatography and Mass Spectroscopic analysis of crude methanolic extract was done. The compound obtained from GC-MS were identified with compare with mass spectral analysis of the moss *Bryum coronatum* (Fig. 24. chromatogram) and also revealed the presence of compounds like Butanal, 2-ethyl-3-methyl-, Undecane,3-Allyl-6-methoxyphenol, Methyleugenol, 2,4-Di-tert-butylphenol,Diethyl Phthalate, Tetradecanoic acid, Decane, 1-iodo-Hexadecanoic acid, methyl ester, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dienn-Hexadecanoicacid,Dibutylphthalate, 1-Heptacosanol,11,14-Eicosadienoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, Oleic Acid, Octadecanoic acid, Hexadecanoic acid, 2-hydroxy-1-(hydroxymet, 4,4'-((p-Phenylene) diisopropylidene) diphenol, Octadecanoicacid, 2,3-dihydroxypropyl ester and 13-Docosenamide, (Z)-.

The chemical components from the methanolic extract of *Bryum coronatum* was identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Asakawa (1981, 1984) reported the presence of terpenoids and lipophilic aromatic compounds in liverworts as potent source of antibiotics. By using Gas Chromatography and Mass Spectroscopy (GC-MS) techniques compounds like monoterpenoids, sesquiterpenoids,

diterpenoids, bicarbocyclic diterpenoids, triterpenoids, phenolic compounds, sterols, flavonoids, and fatty acids can be found out (Banerjee, 2001). Wankhede *et al* (2005) trace the possible chemical compounds, from crude methanolic extract of Plagiochasma appendiculatum by Gas Chromatography and Mass Spectroscopic analysis . They revealed the presence of compounds like Caryophyllene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoicacid, Phytol, Hexacosane and Heneicosane.

Muthulakshmi *et al*., (2012). Studies on biologically active compounds in bryophytes are rapidly growing, resulting in identification of a large number of specific substances with high biological activity. Asakawa *et al.,* (2013) find out that bryophytes emit simple aromatic volatile terpenoids which are responsible for intense mushroomy, sweet mossy, sweet woody, carrot like adour or seaweed. GC-MS GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc.

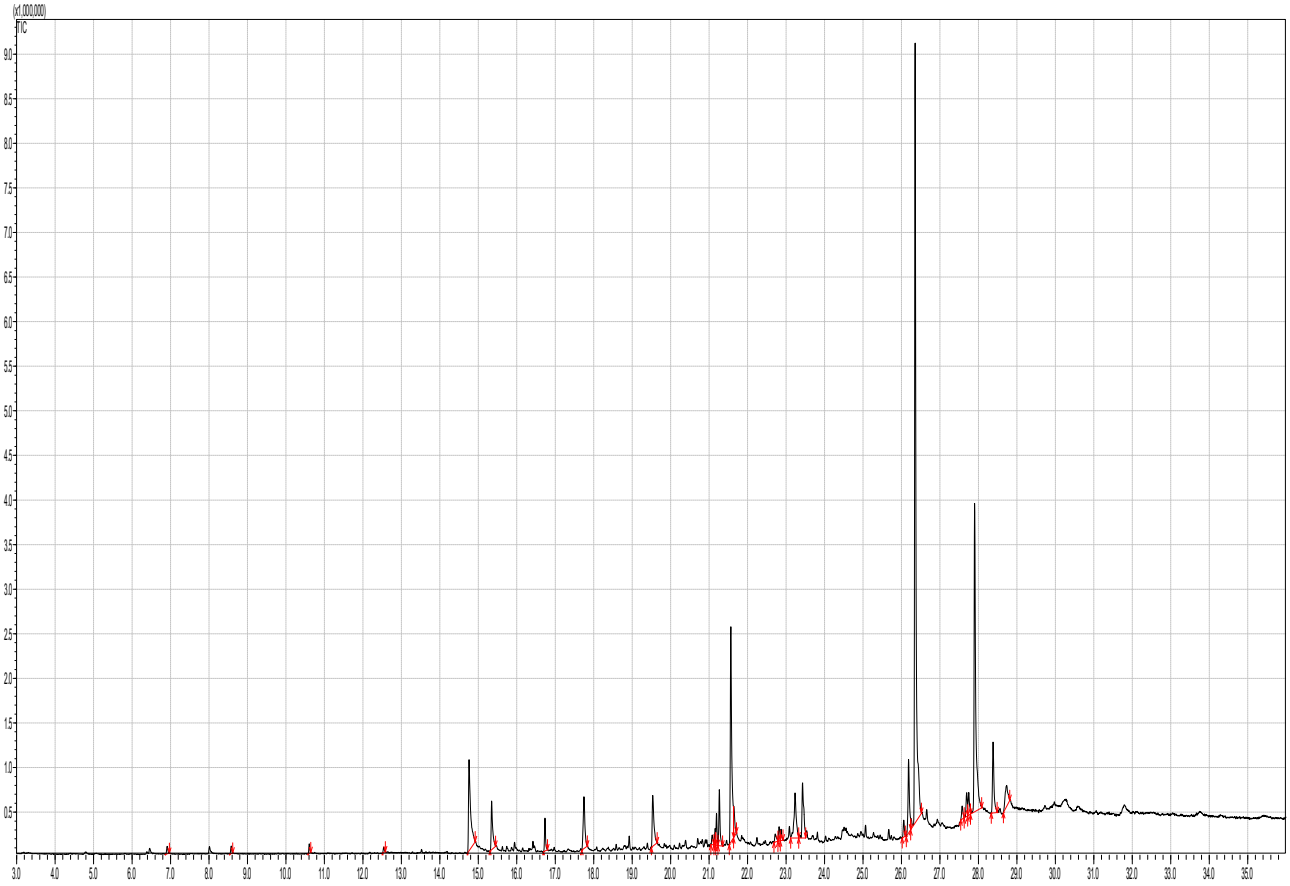
Table. 24- GC-MS chromatogram of *Bryum coronatum* Schwager

Peak report of TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	6.912	179612	0.28	82495	0.35	Butanal, 2-ethyl-3-methyl-

2	8.578	159392	0.25	85189	0.36	Undecane
3	10.606	174782	0.28	105904	0.44	Undecane
4	12.542	123096	0.19	60119	0.25	Unmatched
5	14.760	3794771	5.97	1023541	4.29	3-Allyl-6-methoxyphenol
6	15.347	1453013	2.29	546666	2.29	Methyleugenol
7	16.732	718105	1.13	369018	1.55	2,4-Di-tert-butylphenol
8	17.746	1777020	2.80	582351	2.44	Diethyl Phthalate
9	19.534	1782780	2.81	568814	2.38	Tetradecanoic acid
10	21.075	268583	0.42	104102	0.44	Unmatched
11	21.155	354836	0.56	185876	0.78	Decane, 1-iodo-
12	21.192	674433	1.06	358379	1.50	Hexadecanoic acid, methyl ester
13	21.267	1288367	2.03	626765	2.63	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien
14	21.564	6280517	9.89	2415060	10.12	n-Hexadecanoic acid
15	21.645	661411	1.04	345453	1.45	Dibutyl phthalate
16	22.710	294405	0.46	83568	0.35	1-Heptacosanol
17	22.820	382460	0.60	162011	0.68	11,14-Eicosadienoic acid, methyl ester
18	22.870	247003	0.39	128956	0.54	9-Octadecenoic acid (Z)-, methyl ester
19	23.233	2031795	3.20	506731	2.12	Oleic Acid
20	23.429	2006866	3.16	616549	2.58	Octadecanoic acid
21	26.059	383690	0.60	188340	0.79	Unmatched
22	26.186	2213275	3.48	823617	3.45	Unmatched
23	26.354	21368529	33.64	8745987	36.64	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
24	27.577	361645	0.57	146207	0.61	Unmatched
25	27.694	632151	1.00	264931	1.11	Unmatched
26	27.750	668639	1.05	254280	1.07	4,4'-((p-Phenylene)diisopropylidene)diphenol
27	27.901	9909592	15.60	3459673	14.49	Octadecanoic acid, 2,3-dihydroxypropyl ester
28	28.382	2096516	3.30	793988	3.33	13-Docosenamide, (Z)-
29	28.734	1224578	1.93	237074	0.99	Unmatched
		63511862	100.00	2387164 4	100.00	

Figure: 14- GC-MS Chromatogram of *Bryum coronatum* Schewager



4.6 Characterization of selected moss extracts.

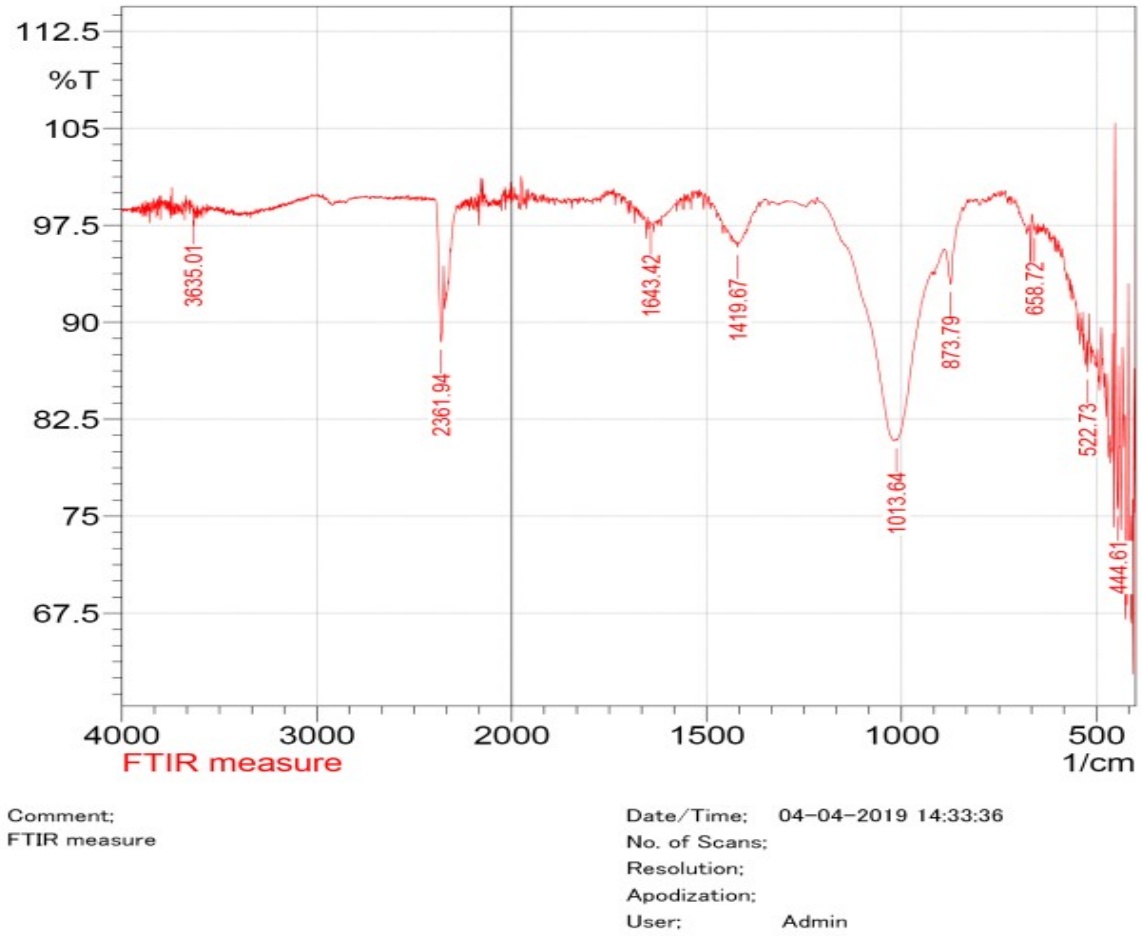
4.6.1. Spectroscopic characterization (FT-IR)

FT-IR spectra of extracts showed the normal stretching frequency for alcohols at 3635.1 cm^{-1} and characteristic stretching bands of C=N in 2361.94 cm^{-1} region indicating the nitrile group. FT- IR spectra exhibits the intensive band of C = C group at 1643.42 cm^{-1} and characteristic bending bands of C = O in 1419.67 cm^{-1} region indicating the carbonyl groups of phenolic esters. The spectra showed the C-O stretching bands of ethers and alcohols at 1013.64 cm^{-1} and bending at 873 cm^{-1} for aromatic compounds. The intensities of absorption bands in 522.73 – 658.72 cm^{-1} region exhibits stretching frequency for alkyl halides (R-x). FT- IR spectra of extracts showed the normal stretching frequency for alkene = C-H bending at 444.61 cm^{-1}. Thus the ethanolic extract of *Bryum coronatum* showed considerably higher concentration of phenolics (1643.42 cm^{-1}).

Table 25. Assignment of major spectral bands in the Fourier transform infrared spectra of *Bryum coronatum* Schwager

Wave number, cm–1	FT-IR band assignments and main origins.
3635.1	Normal stretching frequency, mainly for alcohols
2361.91	Stretching bands of C=N indicating nitrile group
1643.42-1419.67	Shows intensive band of C=C Group and bending bands of C=O indicates the carboxyl group of phenolic esters.
1013.64	C-O Stretching bands of ethers and alcohols.
873	Bending frequency for aromatic compounds.
658.72-522.73	Stretching frequency for alkyl halides (R-x)
444.61	=C-H normal stretching frequency for alkene

Fig: 15 FT-IR absorption spectra of *Bryum coronatum* Schwaegr methanolic extract.



4.6.2. Characterization by using parameters

A. Susceptibility of target organisms to fresh and stored extracts *Bryum coronatum* Schwaegr: This test shows fresh and stored extract of *Bryum coronatum* Schwaegr, there is no

change in *Fusarium oxysporum* and *Penicillium notatum* and slight change in *Aspergillus niger* and *Trichoderma viride*. (Table – 21)

B. Effect of pH on activity and stability of bioactive components from *Bryum coronatum* Schwaegr extract:

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 *Aspergillus niger*, *Fusarium oxysporum, Penicillium notatum* and *Trichoderma viride* 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, whereas the activity decreased at pH 7.3 and bellow. **(**Table22)

C. Thermal stability of antibiotic components of *Bryum coronatum* Schwaegr extracts:

Since the activity of bioactive extracts was quite stable at varied temperature. Experiments were conducted to see the effect of raised 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. (Table – 23)

Reports on traditional use of bryophytes as medicine are available (Harris, 2008) but very limited attempts have been taken to exploit the medicinal, clinical and pharmacological, potential of bryophytes. Traditional use of bryophytes when scientifically confirmed, might serve an exciting aspect to explore the enormous bioactive potential of this plant group. From centuries, higher plants have been used for this purpose. But the present attempt was made with respect to mosses of lower group of plants.

Table 26: Susceptibility of target organisms to fresh and stored extracts *Bryum coronatum*

Sr. No.	Nature of extract	Inhibition zone (mm) against target organisms			
		A	B	C	D
1	Fresh extract	0.7	0.7	0.8	0.8
2	Stored extract	0.6	0.7	0.8	0.7

A. *Aspergillus niger* (NFCCI 3114) B. *Fusarium oxysporum* (NFCCI 1276).
C. *Penicillium notatum* (NFCCI 1072) D. *Trichoderma viride* (NFCCI 1139).
Data values represent average of three replicates.

Table27: Effect of pH on activity and stability of bioactive components from *Bryum coronatum* extract:

Sr. No.	pH	Inhibition zone (mm) against target organisms			
		A	B	C	D
1	5.8	11	NI	NI	NI
2	6.8	12	NI	9.0	NI
3	7.3	11	NI	9.5	NI
4	8.0	11	NI	8.0	NI

A. *Aspergillus niger* (NFCCI 3114) B. *Fusarium oxysporum* (NFCCI 1276).
C. Penicillium notatum (NFCCI 1072) D. *Trichoderma viride* (NFCCI 1139).
Data values represent average of three replicates.

Table 28: Thermal stability of bioactive components of *Bryum coronatum* extracts:

Sr. No.	Temperature (°C)	Inhibition zone diameter (mm) against target organisms	
		A	B
1	30	13	10
2	40	12	9
3	50	12	9
4	60	11	8

A. *Aspergillus niger* (NFCCI 3114) B. *Fusarium oxysporum* (NFCCI 1276).
Data values represent average of three replicates.

5. Summary and conclusion

The present work mainly includes the studies on the antimicrobial properties, isolation and characterization of metabolites of mosses from Western Ghats of Maharashtra. Chapter one gives idea about the work done by different bryologist. It also explains the need of the present

work, to be carried out in Maharashtra. It describes the reasons which promoted the author to carry out the present research work.

Chapter two deals with review of literature with reference to antifungal and antibacterial activities of mosses at global and India level. Mycoflora of rhizosphere and non rhizosphere soils, phytochemical analysis of mosses, application potential of mosses and their sec-metabolites and research limitations of mosses.

Chapter three gives idea about material and methods of the work carried out during the course of investigation. The survey of mosses undertaken from different localities of Western Ghats of Maharashtra. (MAP-1). It includes selection of sites, collection, separation, storage and identification of mosses during July 2015 to Sept 2018.

For analysis of physico-chemical and biological characteristics, soil samples are separated from mosses and also collected from different localities. Elemental analysis is carried at soil testing Laboratory of Daund sugar pvt Ltd, Alegaon. Tal. Daund. Dist. Pune and Krushi Vidynan Kendra, Shardanagar, Baramati. Dist. Pune.

The chapter also describes the screening process for isolation of fungi / mycoflora of rhizosphere and non-rhizosphere soils, antimicrobial screening of 10 mosses, preparation of extraction with different organic solvents and distilled water.

The plant extracts are subjected for antifungal and antibacterial activities through 'Disc diffusion assay method'. The cultures are obtained from National Fungal Culture Collection of India, (NFCCI), Agharkar Research Institute Pune. India. These included four bacterial strains viz. *Bacillus substilis* (NFCCI 2697), *Escherichia coli* (NFCCI 2067), *Pseudomonas aeruginosa* (NFCCI 2200), *Staphylococcus aureus* (NFCCI 2492) and four fungal strains viz. *Aspergillus niger* (NFCCI 3114), *Fusarium oxysporum* (NFCCI 1276), *Penicillium notatum* (NFCCI 1072), and *Trichoderma viride* (NFCCI 1139). Qualitative analysis for alkaloids, glycosides, tannins, steroids, saponins, phenols, terpenoids and flavonoids was carried out by phytochemical tests. HP-TLC and FT-IR are carried out for confirmation of secondary metabolites. Bioactive fresh and stored extracts are characterized for susceptibility of target organisms. The extracts are subjected for analysis of effect of PH and temperature.

In chapter four, result and discussion is major part. It gives detailed information about identified moss species and its comparison with the preserved herbarium voucher specimens stored at laboratory. The selected 10 species of mosses belonging to 9 genera, distributed over 8 families. The collected mosses are arranged systematically according to the classification given by Chopra (1958). It provides key for identification of mosses. Identified mosses are used for physico-chemical and biological characteristics of soil, antimicrobial screening and isolation and characterization of metabolites.

In this chapter, the physical properties like soil color, texture, structure, soil composition, electrical conductivity and P^H from each locality is documented. Purandar locality, soil contains vary large amount of fine particles, especially clay. On the other hand, laterite soil at Kaas-Satara, red soil from Lonawala, light black from Khandala, reddish brown at Sinhagad, yellow brown at Mahabaleshwar and Pachgani, brown and light brown soil observed at Aundh and Bhimashankar.

The chemical analysis of soil made for available nitrogen (N), phosphorous (P) potassium (K) and organic carbon % (C %). Soil samples are categorized as rhizosphere and non – rhizosphere. The fungal isolation was made by serial dilution method and identified by using standard literature.

The significant findings of the present study are summarized as follows:

1. The mosses grow on different habitats such as on wait walls, moist soils, rock surfaces, crevices of rocks and on bark of trees. Maximum number of moss species were found at Lonawala. *Hyophila involuta* (Hook) Jaeg. and *Bryum coronatum* Schwager,are terrestrial mosses. Mosses like *Hypnum reflexum* F. E. Tripp, *Steeriophyllum ancens* (Bosch et Lac.) Broth, *Fissidens crenulatus* Mitt and *Trachypodiopsis blanda* (Mitt.) Fleisch are epiphytic. *Funaria hygrometrica* Hedw grow on moist rocks.

2. Mosses collected from 10 localities showed that they grow at higher altitude, rainfall and humid conditions. The altitude ranges from 550 m to 1438 m. The average rainfall varied from 534 mm to 6498 mm. Average humidity varied from 30% to78.5%.

3. Non rhizosphere soil analysis showed that soil was rich in inorganic matter. Nitrogen, Phosphorous and Potassium ranges from 133 - 623, 1.39 - 47.3, 114.10 -742, mg /100 gm. respectively and C % 0.23 - 3.91. Soil pH ranges from 6.2 to 7. 35. the most of the localities are acidic, only two localities are alkaline in nature. Electrical Conductivity (EC) of different localities was ranging from 0.19 to 7.64 dS /m. Sandy clay, sandy loam, course sand, clay loam and loam sandy soils are reported from the studied localities.

4. Soil analysis showed that mosses like *Steeriophyllum ancens* and *Macromitrium sulcatum* grow in saline condition. Also, low EC values are indicating of less free ion availability and poor soil condition.

5. The values of micronutrients of non-rhizosphere soil varies from species to species. Iron content was comparatively higher in all studied mosses. Micronutrients in moss species showed a general trends of decrease in quantify Fe> Mn> Zn>Cu.

6. Rhizosphere and nonrhizosphere soils were analyzed for fungal isolation and its identification. During investigation 12 fungal isolates were obtained from the rhizosphere soil samples and were identified. The identified soil fungi are *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus unguis, Candida albicans, Fusarium oxysporum, Glomus fasciculatum, Penicillium aurantiogriseum P. chrysogenum, Penicillium islandium. Rhizopus stolonifer* and *Trichoderma citrinoviride.*

Among the identified species the fungus *Aspergillus niger* was dominant and followed by *Penicillium chrysogenum.*

7. From nonrhizosphere soil only 5 fungal isolates were obtained and identified. The identified soil fungi are *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Rhizopus stolonifer* and *Fusarium oxysporum.*

8. Antifungal screening of *Bryum ghatens* and *Hypnum reflexum* methanolic extracts indicates the greater inhibitory activity against *Fusarium oxysporum. Funaria hygrometrica* showed antifungal activity against all test fungi. *Steeriophyllum ancens* showed maximum antifungal activity against *Penicillium notatum.*

9. Ethanol extracts of *Hypnum reflexum* showed maximum antifungal activity against all test fungi. *Hyophila involute and Bryum ghatens* showed antifungal activity against *Fusarium oxysporum.*

10. Petroleum ether extracts of *Funaria hygrometrica* showed moderate antifungal activity against *Penicillium notatum* followed by *Hyophila involute* against *Fusarium oxysporum* and *Trichoderma viride.* No inhibition zone was occurred for *Bryum turgidium*, *Bryum coronatum, Hypnum reflexum, Trachypodiopsis blanda* and *Funaria hygrometrica* extracts for all tested fungi. *Brachymenium turgidum* does not showed antifungal activity against *Aspergillus niger, Penicillium notatum* and *Trichoderma viride.*

11. Aqueous extract of *Hypnum reflexum* shows antifungal activity against all test fungi and maximum for *Trichoderma viride. Bryum ghatens, Fissidens crenulatus* and *Trachypodiopsis blanda* did not show antifungal activity against *Fusarium oxysporum* and *Penicillium notatum.*

12. Methanolic, ethanolic and aqueous extract of *Hypnum reflexum* showed strong antifungal activity against all test fungi.

13. Methanolic extract of *Bryum coronatum and Bryum ghatens* showed maximum antibacterial activity against *Pseudomonas aeruginosa. Funaria hygrometrica* and *Bryum coronatum* shows antibacterial activity against all test bacteria. *Hypnum reflexum* showed antibacterial activity against *Bacillus substilis.*

14. Ethanolic extracts of *Bryum coronatum, Hyophila involute* and *Macromitrium sulcatum* showed antibacterial activity against *Escherichia coli. Macromitrium sulcatum* also showed antibacterial activity against *Bacillus substilis.* However *Fissidens crenulatus* did not show inhibition zone against *Pseudomonas aeruginosa* and *Escherichia coli.*

15. Petroleum ether extracts of *Macromitrium sulcatum* showed maximum antibacterial activity against *Escherichia coli. Fissidens crenulatus and Trachypodiopsis blanda* showed antibacterial activity against Bacillus substilis. *Funaria hygrometrica Hypnum reflexum and Steeriophyllum ancens* does not show inhibition zone against all test bacteria.

16. Aqueous extract of *Trachypodiopsis blanda* show maximum antibacterial activity against *Pseudomonas aeruginosa*. *Trachypodiopsis blanda* did not show inhibition zone against test bacteria except *Bacillus substilis*.

17. In general methanolic and ethanolic extract of *Bryum coronatum* showed more antibacterial activity as compared to petroleum ether and aqueous extract. Petroleum ether extracts of *Macromitrium sulcatum* showed maximum antibacterial activity

18. Preliminary screening of secondary metabolites of *Bryum coronatum* extract ensured the presence of flavonoids, steroids, phenols and phytochemicals such as alkaloids, tannins, terpenoids, Saponins and glycosides are not detected in it.

19. HPLC techniques revels the presence of quercetin and other components such as thymol, piperine and ferulic acid.

20. GC-MS analysis of methanolic extract of *Bryums coronatum* revealed the presence of compoundslike Butanal, 2-ethyl-3-methyl-, Undecane, 3-Allyl-6-methoxyphenol, Methyleugenol, 2,4-Di-tert-butylphenol,Diethyl Phthalate, Tetradecanoic acid, Decane, 1-iodo-Hexadecanoic acid, methyl ester, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dienn-Hexadecanoic acid, Dibutyl phthalate, 1-Heptacosanol,11,14-Eicosadienoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, Oleic Acid, Octadecanoic acid, Hexadecanoic acid, 2-hydroxy-1-(hydroxymet, 4,4'-((p-Phenylene) diisopropylidene) diphenol, Octadecanoic acid, 2,3-dihydroxypropyl ester and 13-Docosenamide, (Z)-Some of these compounds shows biological activity.

21. FT-IR spectra of extracts showed the normal stretching frequency for alcohols at 3635.1 cm^{-1} and characteristic stretching bands of C=N in 2361.94 cm^{-1} region indicating the nitrile group. FT- IR spectra exhibits the intensive band of C = C group at 1643.42 cm^{-1} and characteristic bending bands of C = O in 1419.67 cm^{-1} region indicating the carbonyl groups of phenolic esters. The spectra showed the C-O stretching bands of ethers and alcohols at 1013.64 cm^{-1} and bending at 873 cm^{-1} for aromatic compounds. The intensities of absorption bands in 522.73 – 658.72 cm^{-1} region exhibits stretching frequency for alkyl halides (R-x). FT- IR spectra of extracts showed the normal stretching

frequency for alkene = C-H bending at 444.61 cm^{-1}. Thus the ethanolic extract of *Bryum coronatum* showed considerably higher concentration of phenolics (1643.42 cm^{-1})

22. In fresh and stored extracts of *Bryum coronatum*, there is no change in activity of bioactive compounds for *Fusarium oxysporum* and *Penicillium notatum* and slight change in antifungal activity for *Aspergillus niger* and *Trichoderma viride.*

23. The bioactive extract was quite stable within this pH range as tested against target organisms such as *Aspergillus niger*, *Fusarium oxysporum, Penicillium notatum* and *Trichoderma viride* after incubation of bioactive extracts at pH range of 5.8 to 8.0, the maximum antifungal activity was observed at pH range of 6.8 to 8.0, whereas the activity decreased at pH 7.3 and below. The bioactive extracts was quite stable at different temperature range as tested against target organisms.

Conclusions:

- The present research is based on occurrence and taxonomy of mosses at different localities differing in altitude, rainfall, humidity and substratum. Maximum number of moss species found at Lonawala and Mahabaleshwar. The dominant species are *Fissidens crenulatus* Mitt. *Funaria hygrometrica* Hedw., *Macromitrium sulcatum* Brid. and *Hydrogonium arcuatum* is rare species.

- The chemical analysis of major nutrient content in various non rhizosphere soil revels that soils are rich in, Phosphorous and Potassium. The most of the localities are acidic only two localities are alkaline in nature.

- Rhizosphere soils are rich in mycoflora as compared to non rhizosphere soils. Among the identified species the fungus *Aspergillus niger* was dominant followed by *Penicillium chrysogenum.*

- The antimicrobial test results revealed that *Bryum coronatum* and *Steeriophyllum ancens* extracts had a potential activity against all test microorganisms, except *Fusarium oxysporum* and *Aspergillus niger.* The highest antifungal effect was shown in methanol extracts, aqueous showed the lowest level of antifungal effect.

- The results showed that *Trichoderma viride* and *Penicillium notatum* were found to be more sensitive than the studied test strains. *Staphylococcus aureus* was sensitive against *Steeriophyllum anceps* extracts, this strain was resistant against *Bryum coronatum* and *Hypnum reflexum* extracts. *Escherichia coli* was resistant against *Hyophila involuta.*

- Methanol, ethanol, petroleum ether and aqueous extracts showed significant antifungal and antibacterial activities against almost all the test organisms.

- Among the tested bacteria *Pseudomonas aeruginosa* was found to be most sensitive. *Escherichia coli* come next, followed by *Bacillus substilis*.

- These mosses samples showed inhibition effect against both the gram positive and gram negative bacteria. However antibacterial activity of mosses was found to be active against gram negative bacteria. This makes the advantage of selected mosses as antibacterial

agent. Further is being carried out for isolation of bioactive chemical constituents from the active fraction and their mode of action on microbes.

- Present work will provide a comparative study of sensitivity of bacteria and fungi against mosses which indicates that the mosses are rich source of antibacterial and antifungal substances. The observations of this work will play key role for biological control of microorganisms.

- Preliminary screening of secondary metabolites of *Bryum coronatum* extract ensure the presence of flavonoids steroids and phenols.

- *Bryum coronatum* extracts showed the presence of nitrile group, carbonyl groups of phenolic esters, ethers and alcohols, aromatic compounds, alkyl halides and alkene. Thus the ethanolic extract of *Bryum coronatum* showed considerably higher concentration of phenolics.

- Isolation and characterization of secondary metabolites of such compounds may lead to the introduction of new active compounds for possible application in pharmacy after further pharmacological tests.

- Methanolic extract of *Bryum coronatum* Schwaegr, does not showed any change in activity of bioactive compounds by changing pH and temperatures range as tested against target organisms. The bioactive compounds was stable at different temperatures.

- The obtained results showed that mosses may be used as possible natural antimicrobial agents to control various human, animal and plant diseases. Generally it is known that natural compounds are better than synthetic material.

- The present work is based on original research. Almost care has been taken to confirm the results and have been confirmed through several replications. Our results revealed that the selected mosses might possess a novel antimicrobial agents. This study help in the discovery of new antibiotics that could serve as selective agents for infectious diseases which can be used as natural antibiotic agents with proper scientific validation. Further research is needed to obtain information about correlation between chemical composition and antimicrobial activity of moss species.

- Of the mosses investigated, the inhibition effect seen against 4 bacteria and 4 fungus by *Bryum coronatum* extracts suggests that it may be used as a broad spectrum antibiotic in the future. The presence of a specific group (flavonoid and terpenoids) of compounds in extracts might be the reason for this observation. *Hypnum* the extracts demonstrated especially antibacterial activity against *B. subtilis, S. aureus and P. aeruginosa.* These results indicate that the extracts investigated should find a practical application in the prevention of and protection against both gram (+) and gram (-) bacterial infections in plants, animals and humans.

- Further research is being carried out on the isolation of bioactive chemical constituents from the active fractions and their mode of action on microbial cells.

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