"PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL ACTIVITY OF SECONDARY METABOLITES FROM SELECTED SPECIES OF BRYOPHYTES AND PTERIDOPHYTES"

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By

MS. CHELSEA LORRAINE MASCARENHAS

Under the guidance of

DR. RUPALI BHANDARI

DEPARTMENT OF BOTANY

GOA UNIVERSITY

Taleigao Plateau

Goa-403206

APRIL/MAY, 2022

DECLARATION

I hereby declare that this dissertation entitled "**Phytochemical analysis, Antioxidant activity and Antimicrobial activity of secondary metabolites from selected species of Bryophytes and Pteridophytes**" is an authentic work done by **Ms. Chelsea Lorraine Mascarenhas**, student of M.Sc., Botany, Goa University, in partial fulfilment of the requirement of Master of Science in Botany for the University and no part thereof has been presented before any other degree or diploma in any university.

Date: Signature of Student

(**Ms. Chelsea Lorraine Mascarenhas**)

CERTIFICATE

It is certified that the dissertation entitled "**Phytochemical analysis, Antioxidant activity and Antimicrobial activity of secondary metabolites from selected species of Bryophytes and Pteridophytes**" submitted by **Ms. Chelsea Lorraine Mascarenhas** in partial fulfilment for the degree of Master of Science in Botany of Goa University is an authentic record of the dissertation carried out by her under my supervision and guidance.

Date: Signature of Guide

(**Dr. Rupali Bhandari**)

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ABBREVIATIONS

F- *Funaria hygrometrica*

C- *Cyathodium tuberosum*

P- *Pteris vittata*

A- *Adiantum lunulatum*

TPC - Total Phenolic Content

GAE - Gallic Acid Equivalent

ROS - Reactive Oxygen Species

DPPH - 2, 2-diphenyl- 1-picrylhydrazyl

IC_{50} - Half-maximal inhibitory concentration

MEA - Malt-Extract Agar

PDA - Potato Dextrose Agar

TLC - Thin Layer Chromatography

R_f - Retention Factor

UV-VIS - UV–Visible Spectrophotometry

Chapter 1

INTRODUCTION

Chapter 2

MATERIALS AND METHODS

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ABSTRACT

Three different extraction solvents (methanol, ethanol and acetone) were used to extract the bioactive components like alkaloids, total phenolic, flavonoids, saponins, steroids and tannins from Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*). Carbohydrates and proteins are the phytochemical compounds present in methanolic, ethanolic and acetone extracts of Bryophytes whereas, carbohydrates, glycosides, saponins, proteins, fixed oils and fats and phenolic compounds are the phytochemical compounds present in methanolic, ethanolic and acetone extracts of Pteridophytes. The quantity of Total Phenolic Content (TPC) in methanolic and ethanolic extract of *Pteris vittata* and acetone extract of *Adiantum lunulatum* was higher as compared to Bryophytes. The antioxidant activity correlated with Total Phenolic Content (TPC). The methanolic and ethanolic extract of *Pteris vittata* and acetone extract of *Adiantum lunulatum* showed a higher antioxidant activity as compared to Bryophytes. For antifungal activity, more inhibition of growth of the fungal (*Aspergillus* sp.) strain was observed in methanolic, ethanolic and acetone extracts of Pteridophytes as compared to Bryophytes. For antibacterial activity, less inhibition of growth of the bacterial (*Escherichia coli*) strain was observed in methanolic, ethanolic and acetone extracts of Pteridophytes. The Phenolic compounds, Flavonoid compounds and photosynthetic pigments were tentatively identified in methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) using TLC. Overall, the study indicates that the methanolic, ethanolic and acetone extracts of Pteridophytes showed better results as compared to Bryophytes. These phytochemical compounds present could offer protective effects against chronic diseases, such as cardiovascular diseases, diabetes, and cancers. They are also responsible for the anti-inflammatory and antimicrobial effects, as well as

other medicinal values. Bioactive compounds isolated from these lower plants could also be used as nutraceutical and pharmaceutical ingredients.

1. INTRODUCTION

1.1. Bryophytes

Bryophytes are autotrophic cryptogams comprising approximately 25,000 species. Taxonomically, they are divided into three classes, the hornworts (Anthocerotopsida), the liverworts (Hepaticopsida) and the mosses (Bryopsida).The life history of bryophytes involves an alternation between sporophytic and gametophytic generations that differ in form and function. The actual plant is represented by the gametophytic generation. The spores germinate to form a branched or thallose protonema which resembles green algae (Zechmeister, Grodzińska, & Szarek-Łukaszewska, 2003).The green gametophore produces the sex organs. After successful pollination, a sporophytic generation evolves which remains attached to the green plant and is nourished by the gametophore. Depending on size, most of the spores released by the sporangium are dispersed by wind, by rising up to several thousand metres they can be transported over large distances (Longton, 1997). Bryophytes are generally small (less than 5 cm) but some can grow up to a length of 70 cm (e.g. *Polytrichum, Dawsonia*) (Mägdefrau, 1982).Bryophytes thrive in humid climates, but can be found all over the world, even in arid regions (Szweykowski, 1984). They colonise nearly every kind of terrestrial substrate (e.g. bare stones, bark, skeletons, etc.) and grow in freshwater but are absent from saline water bodies (salt lakes, oceans) (Longton, 1988; Pòcs, 1982).

Bryophyte cultivation may be required for physiological and biochemical research. More so, some bryophyte species add to the beauty of gardens, front and backyards as well as landscapes. They can be established easily into diverse environments through transplanting or blending their fragments in a blender. Thus, fragmentation is a form of vegetative reproduction in bryophytes. Bryophytes have dioecious and monoecious

representatives on the basis of their sexual mode of reproduction. About 70% of liverworts species are dioecious, 55 - 60% of moss species whereas in hornworts monoecy is dominant (Vanderpoorten & Goffinet, 2009). Reproduction occurs through spores borne on the gametophytes often as a 'headdress'. These spores require water for formation (for the movement of sperms to fertilize the eggs) and wind for dispersal. Spore capsules (a stalk called seta) are produced after the sperm has fertilized the eggs. Fertilization of gametes forms the gametophyte with the spore capsules called sporophyte. Generally, bryophytes can reproduce asexually when sporophytes release spores and sexually when gametes fuse to form a zygote. Frey & Kurschner (2011) suggested that asexual reproduction in bryophyte occur: 1) Dioeciously through regeneration from specialized caducous organs or by the production of specialized propagules like gemmae and protonemal cells. 2) By the fragmentation of their plant body. 3) Clonally (i.e. self-cloning due to the endogenous mechanism or forced cloning due to external influences). That results in ramets (independent daughter plants also called merriments).The process of germination begins in the capsule, mother cells of spores (sporocytes), which split meiotically into tetrads of haploid spores. These are dispersed and germinate into a filamentous phase called protonema with chloronema, caulonema, and Rhizoids cells. In leafy liverworts, the gametophyte possesses rhizoids, caulid (stem), and phyllids (leaves). Sexual reproduction in bryophytes involves the release of motile male gametes into the environment and requires successful navigation of these naked cells from the male to the female sex organs via an external water source.

The economic cost of their roles in erosion control, environmental bioindicators, as material for seedbeds, fuel, medicines and food sources, pesticides, nitrogen fixation, moss gardening, treatment of waste, construction, clothing, furnishing, packing, genetic engineering and for soil conditioning and culturing remain invaluable in

sustainable terms. Due to their high-water holding capacity, bryophytes are used in horticulture as a soil conditioner and additives for cultivation (Saxena & Harinder, 2004). Hornworts form symbiotic relationships with nitrogen fixing bacteria and produce pores that may be homologous to stomata. Peat result when plant matter such as Sphagnum accumulates under waterlogged conditions without completely undergoing decomposition due to lack of sufficient oxygen, appropriate temperatures, nutrients, and pH. This matter can be used as peat fuel and may be harvested/ dugged out in blocks, dried, and burned for heat in Ireland, Russia, Ireland, Finland, Sweden, Germany, United States and Poland. They have also been implicated in agriculture to increase the water-holding capacity and lightens the soil. Physiologists and even medical scientists are realizing the potential of the bryophytes in understanding gene function and in producing needed proteins (Glime, 2017). Bryophytes are good environmental indicators. For instance, mosses are also good indicators of acid rain, because they lack a protective epidermis and cuticle and, hence, are more susceptible than the vascular plants (Saxena & Harinder, 2004). There is limited information on the diverse economic relevance of bryophyte. For instance, Chandra et al. (2017) reported that in spite of their implication in popular herbal and food remedy among the tribal people of Africa, America, Europe, Poland, Argentina, Australia, New Zealand, Turkey, Japan, Taiwan, Pakistan, China, Nepal and India; very limited knowledge is available about the medicinal properties of bryophytes. The most commonly used bryophytes are *Marchantia, Sphagnum, Polytrichum, Conocephalum, Climacium, Hylocomium, Hypnum, Rhytidiadelphus, Thuidium, Antitrichia, Bryum, Dicranum, Fontinalis, Funaria, Philonotis, Pleurozium and Rhizomnium*. From the ancient times, bryophytes were used in packing, plugging as well as in decoration (Chandra et al., 2017). Bryophytes are considered to be nutritionally useless to humans because no references concerning use as foods for humans have been found unlike their use as medicines

(Asakawa et al., 2013). Some bryophytes are attractive to herbivores. Mosses are used for decorative purposes in homes (Saxena & Harinder, 2004). *Marchantia polymorpha* is used in the winery to soaks up the wine and makes a tasty treat (Glime, 2017).Their durability and elasticity may be the reason why they are used to stuff and fill in chinks in wooden buildings, industrial and domestic upholstery, hassocks, between the panes of glass in double-glazed windows, balls, and dolls. Sphagnum is used in America as an absorbent to serves as an insulator to keep warm, dry or cool. Sphagnum has been implicated in making clothes, soap, and ointment for dressing wounds. Tribal people use these plants to cure various ailments in their daily lives including to cure hepatic disorders, skin diseases, cardiovascular diseases, antitumor properties, used as antipyretic, antimicrobial, wound healing, etc. (Chandra et al., 2017). More so, active constituents of bryophytes are widely used as antibacterial, antifungal, cytotoxic, antitumor and insecticidal.

The phytochemistry of bryophytes is not a hot topic because of their very small size and the difficulty associated with their collection and identification (Asakawa et al., 2013). Liverworts contain number of mono-, sesqui- and di-terpenoids, aromatic compounds like bibenzyl, bis-bibenzyls, acetogenins, sesquiterpenes, diterpenes and lipophilic aromatics, which are enantiomers of those found in higher plants that are produced from its cellular oil body (Asakawa et al., 2013). These authors upon investigation verified that these chemical compounds derived from liverworts display a characteristic odor, and can have interesting biological activities including allergenic contact dermatitis, antimicrobial, anticancer, antifungal and antiviral, cytotoxic, insecticidal, insect antifeedant, superoxide anion radical release, 5-lipoxygenase, calmodulin, hyaluronidase, cyclooxygenase, DNA polymerase β, and α-glucosidase. Phytochemical evaluation of bryophytes became popular since the last decades with the use of new methods in gas chromatography, mass

spectrometry, nuclear magnetic resonance, high-performance liquid chromatography, thin layer chromatography and x-ray to isolate and structurally elucidate bioactive molecules present in bryophytes (Dey & Mukherjee, 2015). Phytochemical investigations implicate the presence of biologically active metabolites from carbohydrates, lipid, protein, steroids, polyphenols, terpenoids, organic acids, sugar alcohols, fatty acids, aliphatic compounds, acetogenins, phenylquinones, and aromatic and phenolic (Saxena & Harinder, 2004).

1.2. Pteridophytes

Pteridophytes (ferns and fern-allies) are the most primitive vascular plants that appeared on the Earth, in the mid-Paleozoic era during the Silurian period which began 438 million years ago. They are the earliest of the plants ever evolved on the earth heralding the presence of a well-developed vascular system –xylem for water and phloem for food transport respectively and hence, are referred as 'vascular cryptogams'(Dudani, et al., 2014).Currently about 12,000 species are known. Taxonomically, they are divided into four classes, Psilopsida, Lycopsida, Sphenopsida and Pteropsida. This interesting group of plants form an important component of forest ecosystem and act as connecting bridge between then on-vascular cryptogams and the seed plants and occupy various niches on the land, in marshes, swamps and in water bodies (Dudani, et al., 2011).Pteridophytes are characterized by a life cycle that usually involves an alternation of two free-living generations – sporophyte and gametophyte, with sporophyte being the larger phase of the life cycle. Sporangia are produced on the leaves of sporophytes (sometimes in specialized cone-like strobili).In true ferns, these are commonly on the leaf under surface and are often clustered into discrete units called sori. At maturity, the sporangium dries and ruptures dispersing the spores into the air. Gametophytes are often moss-like in appearance and are

quite small, usually less than 1 cm wide at maturity, but are often fairly easily located in nature near adjacent sporophytes (Yatskievych, 2002).The major centres for Pteridophytes diversity are Eastern and Western Himalayas, Western Ghats, Eastern Ghats, Central India and Andaman and Nicobar Islands. The majority of them thrive well in shady and moist places but a few survive in rock crevices and dry places while some of them such as *Salvinia* and *Azolla* grow in aquatic habitats (Bower, 1923).

Many pteridophytes supplement their sexual cycles with various forms of vegetative reproduction. This may be as simple as the fragmentation of a creeping rhizome into smaller pieces that become established as separate plants. Horsetails (Equisetaceae) growing along rivers and streams are frequently spread over long distances in this fashion by flooding. Other species develop specialized structures to effect vegetative propagation. Some ferns produce stolons, which are specialized long, spreading stems that root at their tips and form new plants. Others produce buds or bulbils on their leaves that can germinate to form new plantlets. Still others produce roots where their fronds come into contact with soil. A few species produce specialized underground structures, such as tubers and similar offsets. Some species have sporophytes or gametophytes that produce gemmae, which are specialized relatively undeveloped fragments of plants that break off and are dispersed, eventually germinating to form new plants. Other ferns and fern allies, including spike mosses (Selaginellaceae), quillworts (Isoetaceae), and aquatic ferns (Azollaceae, Marsileaceae, Salviniaceae), depart from the typical life cycle in producing two different types of sporangia. One of these produces numerous microscopic microspores that germinate to produce male gametophytes. The other sporangial type produces many fewer and much larger megaspores (usually visible to the naked eye), which grow into female gametophytes. Apogamous ferns, which frequently occur in environments with seasonal extremes of heat, cold and/or drought, avoid the necessity for sex. In the sporangia of such

plants, a mechanism during the series of cell divisions results in the production of spores with the same genetic constitution as the sporophyte plant (meiosis does not result in a reduction in chromosomal ploidy). These ‘diplo spores’ grow into gametophytes that produce new sporophytes directly from meristematic tissues near the notch region. The environmental advantages of apogamy include the faster development of the gametophyte and the release from the requirement of standing water for fertilization to take place. Interestingly, many apogamous ferns continue to produce antheridia with functional spermatozoids, which can be released and fertilize eggs on nearby gametophytes of related sexual species. Once formed, such hybrids are always apogamous and thus able to reproduce themselves.

Relatively few species of pteridophytes are economically important. The best-known current use is horticultural, as garden plants, house plants and specimen plants in conservatories and greenhouses. One species, *Ruhmora adiantiformis* is often called florist’s fern; its finely divided but thick and leathery leaves resist wilting and are used in cut flower arrangements. Another horticultural practice has been the use of chunks of the dense rot-resistant root mantles covering the stems of tree ferns (known as orchid bark) as a substrate for growing orchids and other plants that are epiphytic in nature. A number of ferns have been used in handicrafts. Petioles of some members of the climbing fern family, Schizaeaceae, as well as other groups, are used in some tropical countries for colour designs in basketry and bracelets. *Pteridium* (bracken) leaves have been used to make a green dye. The rhizomes of the tree fern *Cibotium*, which are covered with dense, long, golden hairs, have been fashioned since antiquity into animal statue curios some-times known as ‘vegetable lamb of Tartary’. One group of pteridophytes with an extensive history of use is the clubmosses (Lycopodiaceae). The microscopic spores of these fern allies contain non-volatile oils that made them useful as dry industrial lubricants. Other

uses of the spores have been in flash powder for photography and in fingerprint powder used in forensic investigation. Various ferns are also eaten as food, with the young foliage usually steamed as a vegetable or dried and used as an additive in stews and sauces. Several species are eaten, including *Diplazium esculentum* (which is cultivated for this purpose in parts of Asia), but the commercially most important species in the western hemisphere is *Matteuccia struthiopteris*, the ostrich fern, whose fiddle heads are a common sight in markets of the north eastern United States in late spring. Also *Acrosticum* is used in food preparation in different parts of India. Formerly, *Pteridium aquilinum* (bracken) was quite important in some cuisines, particularly in parts of eastern Asia. However, medical studies have linked this species to stomach cancer and its use has declined. Perhaps the most economically valuable species of pteridophyte is *Azolla*, a genus of tiny floating aquatic ferns. For centuries farmers in parts of eastern Asia are jealously guarded strains of this plant, which they used to inoculate rice paddies in the spring for markedly increased yields. They 'discovered' that hollow chambers in *Azolla* leaves contain symbiotic cyanobacteria (*Anabaena azollae*) that are able to convert atmospheric nitrogen into the nitrate form that serves as a major plant nutrient. Thus, the fast-growing plants of *Azolla* acted as a living source of fertilizers. A few ferns have had negative economic impacts because of their weediness. Two of the best examples include *Salvinia* and *Pteridium*. *Salvinia molesta* (Kariba weed, giant *salvinia*) is a floating aquatic fern that is weedy throughout the warmer parts of the world. In some situations it can form a mat several inches thick on the surface that prevents light and oxygen penetrating into the water. *Pteridium aquilinum*(bracken) is a coarse fern with an immense creeping rhizome capable of reaching lengths of 400 m. The plant quickly invades open habitats, competing vigorously with other plants. Because the plants are toxic to livestock, bracken has ruined the pasturage on large acreages of land, especially in parts of Europe.

Ferns have historically been used extensively by humans as ornamental plants, in domestic utensils, in handicrafts, as components of cosmetic formulations and foodstuffs, and for medicinal purposes. Reports of therapeutic effectiveness, as well as scientific curiosity and the need for new drugs have prompted several groups to conduct pharmacological research on ferns and related plants. Bioactive compounds present in pteridophytes include luteolin-7-O-glucoside, 16-hydroxy-kaurane-2-β-d-glucoside, luteolin, palmitic acid, apigenin 4-O-α-l-rhamnoside, quercetin, hyperin, isoquercitrin, kaempferol, rutin (Wang et al., 2010), and apigenin-7-O-β-d-glucoside. p-coumaric, p-hydroxybenzoic, caffeic, ferulic, vanillic, protocatechuic acids Hydroxycinnamic acid, anthocyanins, total polyphenols, and flavonoids. Pharmacological and ethno-pharmacological studies have revealed that substances in ferns exhibit diverse pharmacological effects such as cytotoxicity, hepatoprotective activity, antihyperglycemic activity, leishmanicidal activity, trypanocidal activity, anti-nociceptive activity, anti-inflammatory activity, immunomodulatory activity, antioxidant, antidiabetic, anti-cancer, antiviral, wound healing, antimicrobial, anti-Alzheimer activity and chemopreventive effects. Because of the need for new medicines with different biochemical activities, pteridophytes and their secondary metabolites could potentially be of great medicinal value.

1.3. Phytochemical Studies

Plants has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from plants, many based on their use in traditional medicine (Cragg & Newman, 2013). In fact, plants produce a varied range of bioactive molecules, making them a rich source of dissimilar types of medicines (Singh &

Srivastava, 2013).A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda (Murugan & Saranraj, 2011).

Phytochemicals are naturally occurring in plants, leaves and other vegetative parts and roots. These phytochemicals have a role in defense mechanism of plants and in protection of plants from various diseases (Sabovljevic & Sabovljevic, 2008). Plant and plant-based products are the natural sources of different phytochemicals such as phenols, flavonoids, alkaloids, glycosides, lignins, and tannins. Phenols and flavonoids are the most common phytoconstituents of different fruits, vegetables, and medicinal and aromatic plants, which are responsible for antioxidant activities (Scalbert, et al., 2005).

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plants. They are essential to the physiology of plants, because of their involvement in various important functions (growth, structure, defense, pigmentation, lignification etc.). More than 8,000 polyphenolic compounds are identified in various plant species. Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituent and ranged from simple phenolic molecules to highly polymerized compounds (Bravo, 1998). Polyphenols are classified on the basis of the number of phenol rings that they contain the structural elements that bind these rings to one another. They are broadly divided in four classes: phenolic acids, flavonoids, stilbenes and lignans.

1.3.1. Phenolic Acids

Phenolic acids account for about a third of the polyphenolic compounds in our diet and are found in all plant materials, but are particularly abundant in acidic fruits. Caffeic acid, gallic acid, ferulic acid are some common phenolic acids. Phenolic acids are widely dispersed in plant kingdom. They contain two distinguishing constitutive carbon frameworks, hxydroxycinnamic (C_6C_3) and hydroxybenzoic (C_6C_1) structure. The hydroxybenzoic acid content of edible plants is generally low, with the exception of certain red fruits, black radish and onions which have high concentration (Shahidi & Naczk, 1995). The hydroxycinnamic acids are more common than hydroxybenzoic acids and consist chiefly of p-coumaric, caffeic, ferulic and sinapic acids (Han, Shen, & Lou, 2007). Phenolic acids have antioxidant properties due to their high redox potential, which allows them to act as reducing agents and singlet oxygen quencher (Ignat, 2011).

1.3.2. Flavonoids

Flavonoids constitute the largest class of phenolic compounds with more than 3,000 structures. These consist of two aromatic rings bound together by three carbon atoms that forman oxygenated heterocycle (Tsao, 2012). Due to the hydroxylation pattern and variations in the chromane ring (Ring C), flavonoids can be further divided into different sub-groups such as anthocyanins, flavan-3-ols, flavones, flavanones and flavonols (Merken & Beecher, 2000) of which flavones and flavonols are most widely occurring. Individual differences within each group arise from the variation in number and arrangement of the hydroxyl groups and their extent of alkylation and/or glycosylation (Spencer , Mohsen , Minihane , & Mathers, 2008). More than 4,000 varieties of flavonoids have been identified which are responsible for the attractive

colours of the flowers, fruits and leaves. Quercetin, epicatechin, myricetin, catechins etc. are some of the common flavonoids.

Among the bryophytes, almost all liverworts possess beautiful cellular oil bodies which are peculiar, membrane-bound, cell organelles that can contain ethereal terpenoids and aromatic oils suspended in carbohydrates- or protein-rich matrix, while the other two phyla do not. The biological activities of liverworts are due to terpenoids, aromatic compounds and acetogenins which are present in oil bodies of each species. Most compounds found in the liverworts are composed of lipophilic mono-, sesqui- and di-terpenoids and aromatic compounds, such as typical bibenzyls and bis-bibenzyls which have been isolated from the Marchantiaceae and Aytoniaceae in the Marchantiales, Lejeuneaceae, Lepidoziaceae and Plagiochilaceae in the Jungermanniales and Blasiaceae, Pelliaceae and Riccardiaceae in the Metzgeriales. Most sesqui- and di-terpenoids obtained from liverworts are enantiomers of those found in higher plants. Many of these compounds display a characteristic odor, and can have interesting biological activities (Yoshinori , Ludwiczuk, & Nagashima, 2013).

Ferns produce a wide array of secondary metabolites endowed with different bioactivities that could potentially be useful in the treatment of many diseases. However, there is currently relatively little information in the literature on the phytochemicals present in ferns and their pharmacological applications. Phytochemical studies on ferns have revealed that they contain a wide range of alkaloids, flavonoids, polyphenols, terpenoids, and steroids. The structures of these compounds usually differ from those of related secondary metabolites produced by other higher plants, making them a potentially valuable source of chemical diversity (Cao, Chai, Wang, et al., 2017).

1.4. Antioxidant activity

Plants have a large number of bioactive compounds with high antioxidant activity. Unfavourable conditions for plants, such as extreme temperature, drought, heavy metals, nutrient deficiencies, and high salinity, generate high concentrations of reactive oxygen species (ROS), which can cause oxidative stress. To avoid this, cells have a complex antioxidant system with enzymatic and non-enzymatic elements. The molecules of the non-enzymatic system have different action mechanisms, such as enzyme inhibition, chelation of trace elements involved in the production of free radicals, reactive species uptake and activation or increase in protection through other antioxidant defences (Barua, et al. , 2014). Among these molecules, the compounds derived from secondary metabolism, specifically phenolic compounds play a fundamental role against oxidative stress (Pang, 2018). These compounds are known to act as antioxidants not only for their ability to donate hydrogen or electrons but also because they are stable radical intermediates. Phenolic compounds also have protective effects on humans when the plants are consumed as food (Nićiforović, 2010). An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Yamagishi & Matsui, 2011). The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Wu & Li, 2011). Generally, the antioxidant capacity of phenols in plant extracts is effective at low concentrations, and in humans, it is associated with the prevention of cardiovascular disease and cancer (Miliauskas , Venskutonis, & van Beek, 2004). Thus, studies for the determination of the antioxidant activity of the extract of different plant species could contribute to establishing the value of these species as a

source of new antioxidant compounds (Gouthamchandra, Mahmood, & Manjunatha, 2010).

Bryophytes produce a number of secondary metabolites that strengthen these delicate plants with strong antioxidative machinery to cope up with biotic and abiotic stresses. Several bryophytes have been reported to show significant antioxidant activity. Some of these bryophytes possessed very efficient antioxidant enzyme systems, while others showed the presence of diverse kinds of phenolic and flavonoid compounds responsible for free radical scavenging. Studies also revealed that the antioxidant values of liverworts were closer to those of vascular plants. In one such study on the liverwort *Marchantia polymorpha*, antioxidant enzyme peroxidase was characterized which was found to be different from any known peroxidase of vascular plants (Hirata, Ashida, & Mori, 2002). In another study, the extract of *Plagiochasma appendiculatum* showed significant antioxidant activity by inhibiting lipid peroxidation and increasing superoxide dismutase and catalase activity (Singh, et al., 2006).

Pteridophytes are one of the most important plant groups that is rich in antioxidants and have been used significantly and successfully in folk medicine for several years. Pteridophytic antioxidants can neutralize the effect of AOS which are one of the several factors involved in various physiological malfunctioning of living organisms. Various pharmaceuticals formulations and bioinformatic approaches for drug designing in respect of chemical ligand binding and bioactivity assay in vitro as well as in vivo with reference to these antioxidants can relive disorders whose medicines are not yet available such as cancer. The antioxidant composition of Pteridophytic generas also can aid in solving the phylogenetic puzzle and its chemotaxonomic approaches could also contribute in a new and revised system of plant classification. The two medicinally important fern species, *Adiantum* (Pteridaceae) and *Pteris* (Pteridaceae), were studied and observed that

various concentration of leaves and stem extracts of both these ferns has significant antioxidant potential and can be used as good source of natural antioxidant (Shrivastava , Pandey , & Chauhan , 2014). (Choudhury et al., 2017) reported that *Marsilea minuta* (Marsileaceae) showed higher metal chelating activity than *Diplazium esculentum* proving its potential as a natural antioxidant. However both these species showed dose dependent superoxide scavenging activity.

1.5. Antimicrobial activity

Globally, food spoilage caused by microorganisms still widely affects all types of food and causes food waste and loss, even in developed countries. It has been estimated that the yearly losses of global food reach up to 40% due to various factors including spoilage by microorganisms (Gustavsson, et al., 2011). Bacteria, yeast, and molds are the common types of microorganisms responsible for the spoilage of a considerable number of food and food products. Once these microorganisms reach food products, they grow by utilizing the nutrients and produce metabolites that cause food spoilage. Foodborne disease is another pervasive food safety problem caused by consumption of contaminated food products, which has been a significant safety concern to public health (Kirk, et al., 2017). Despite of the proven efficiency of these chemical preservative in prevention and outbreak control of food poisoning diseases, their repeated applications has resulted in the accumulation of chemical residues in food and feed chain, acquisition of microbial resistance to the applied chemicals and unpleasant side effects of these chemicals on human health (Akinyemi, et al., 2006). Because of such concern, efforts have been focused on developing a potentially effective, healthy, safer and natural food preservative.

Microorganisms are available naturally in the surrounding environment; therefore they can easily reach food during harvesting, slaughtering, processing, and packaging (Hatab, et al., 2016). These microorganisms can survive under adverse conditions used in the food preservation such as low temperature, modified atmosphere packaging, vacuum packaging, as well as resist conventional pasteurization. Thus, there is a considerable concern among consumers regarding the risk of using synthetic additives for human health, that led to decrease the use of these chemicals in food preservation (Gyawali & Ibrahim, 2014). Therefore, new eco-friendly methodologies are required to reduce the growth of pathogenic bacteria and prolong the shelf-life of food products, without using chemical preservatives. Many researchers investigated the possible utilization of some plant extracts as effective natural preservatives (Fernández-López, et al., 2005). Medicinal plants contain several phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids, which possess antimicrobial and antioxidant properties (Talib & Mahasneh , 2010). The crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria (Alzoreky & Nakahara, 2003). In addition, it has been reported that the extracts from Chinese chives and cassia can effectively reduce the growth of Escherichia coli and other bacteria during storage of meat, juices, and milk (Mau, Chen, & Hsieh, 2001). In a similar study, (Doddanna, Patel, Sundarrao , & Veerabhadrappa, 2013) investigated the effect of some plant extracts on the growth of *Candida albicans*, the results indicated that the alcoholic extract of curry leaves effectively inhibit the growth of *C. albicans*. (Nzeako, et al., 2006) reported that thyme oil extract could decrease the growth of *C. albicans* and *Pseudomonas aeruginosa*. The understanding of the mechanism of antimicrobial action of medicinal plants extracts is the first step in the optimal utilization of these extracts as natural antimicrobial agents to extend the shelf-life and maintain the food quality.

Bryophytes (liverworts and mosses) are traditionally used in India by different cultural groups for burns, cuts, wounds, and skin diseases, suggesting that they protect the skin and open wounds from microbial pathogens. Several bryophytes, including the most active from the *Bazzania, Conocephalum, Diplophyllum, Dumortiera, Marchantia, Metzgeria, Lunularia, Pellia, Plagiochila, Porella*, *Radula,* and *Riccardia* genera, were reported to have antimicrobial activity. Sacculatal from *Pelliaen diviifolia* showed potent antibacterial activity against *Streptococcus* mutans, lepidozenolide from *Lepidozia fauriana* showed activity against methicillin-resistant *Staphylococcus aureus* (MRSA), and marchantins from many *Marchantia* species showed activity against more than 10 pathogen bacteria (Asakawa,1990). Chlorinated bibenzyls from *Riccardia marginata* exhibited antibacterial effect against *Bacillus subtilis.*

Among all the pteridophytes, taxa from the Pteridaceae, Polypodiaceae, and Adiantaceae exhibited significant medicinal activity. Antimicrobial properties of ferns are remarkable as compared to the higher plants, may be because of the presence of a large number of defensive biochemical compounds. It has been observed that pteridophytes are not infected by microbial pathogens, which may be one of the important factors for the evolutionary success of Pteridophytes. However, researches on the antimicrobial activity of this plant group are still in their infancy. The ferns of "Adiantum group" were found to be particularly active against gram-positive bacteria.

There is currently relatively very little information available on the phytochemicals, antioxidants and secondary metabolites present in bryophytes and pteridophytes and their pharmacological applications. Because of the need for new bioactive compounds with different biochemical activities, bryophytes and pteridophytes could potentially be of great medicinal value. In this study, two bryophytes and two pteridophytes were studied for their phytochemical composition, antioxidant activity and secondary metabolites. Also,

antibacterial and antifungal activity of these lower plants with different extraction solvents was studied.

OBJECTIVES

The present study aims at understanding the effects of different extraction solvents on the antifungal and antibacterial activity of the two bryophytes and pteridophytes that were studied. The qualitative and quantitative analysis of phenolic compounds, flavonoids, antioxidants and plant pigments were studied by TLC and spectrophotometric methods. Also preliminary screening of the phytochemical compounds of Methanolic, Ethanolic and Acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*). The detailed objectives are as follows:

1. To compare the effect of using 3 different extraction solvents (Methanol, Ethanol and Acetone) to extract the active components like alkaloids, total phenolic, flavonoids, saponins, steroids and tannins from Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).
2. Preliminary screening of phytochemical compounds present in methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).
3. To quantitate the amount of Total Phenolic Content in methanolic, ethanolic and acetone extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum.*
4. To investigate the antioxidant activity of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

5. To evaluate the antifungal and antibacterial activity of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) on fungal (*Aspergillus* sp.) and bacterial (*Escherichia coli*) strains.
6. To carry out TLC profiling and UV-VIS spectra for phenolic, flavonoid and Plant pigments in Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*).

2. MATERIALS AND METHODS

2.1. Plant species used

2.1.1. *Funaria hygrometrica*

Funaria hygrometrica Hedw

Family: Funariaceae

Common names: Common Cord-Moss, Little Goldilocks, Golden Maidenhair

Funaria hygrometrica is almost cosmopolitan in distribution. It is found in a wide variety of disturbed habitats such as moist crevices in soil, walls or paving, next to water taps, gutters and drains, on soil in flower boxes and plant pots. In southern Africa, it occurs at altitudes of 20-2550 m (Atherton, et al., 2010). *Funaria hygrometrica* is inconspicuous when sterile (when the more conspicuous sporophyte has not yet developed). The obovate leaves form a bulb-like cluster at the top of the short, 3-8 mm long stem. The leaves are 2-4 mm long with an acute apex and weakly serrate margins. The midrib ends just below, or extends just beyond the leaf apex. The leaf cells are inflated, sub hexagonal to short-rectangular, somewhat narrower at the margin and oblong in the base. The colourful sporophyte is much longer than the stem from which it arises. The yellowish stalk (seta) that bears the capsule is 20-50 mm long. The distinctive capsule is horizontal to pendent, up to 3 mm long, reddish brown, asymmetrical, curved, and more or less pear-shaped with an oblique mouth, with deep longitudinal furrows when dry. The teeth at the capsule mouth are arranged in two rows, with the outer teeth S-shaped and fused at the apex by a lattice disk. The cells of the capsule lid are twisted counter-clockwise. The inflated, hood-shaped calyptra is often seen covering the immature capsules. The spores are yellow-brown and very small (Glime, 2007) (Figure 1).

Uses:

In nurseries and greenhouses, where *Funaria hygrometrica* flourishes in the wet, disturbed conditions, it is sometimes regarded as a weed. This species is widely used in textbooks and biology classes to demonstrate the life cycle of a typical moss (the alternation of two generations: the plant body or gametophyte bearing sexual organs which develop into the spore-bearing sporophyte), possibly because of the abundant, conspicuous sporophytes and its frequent presence in greenhouses (Magill, 1981). In countries of the northern hemisphere, mosses are commonly used in container gardens with bonsai and bonkei. In such gardens, *Funaria hygrometrica* will provide ‘vegetation' of intermediate size, for example between 'mountains' of *Leucobryum* and ‘grass' of *Physcomitrium* or *Bryumargenteum*. It can also be grown in terraria under more moist conditions (Van Rooy, 2001).

2.1.2. *Cyathodium tuberosum*

Cyathodium tuberosum Kashyap

Family: Cyathodiaceae

Cyathodium tuberosum grows in a variety of shaded habitats, along river banks on soil or rocks, on waterfalls, caves, cement floors, stairs and flower pots, and may also be corticolous on bark of several species including *Ficus, Anacardium excelsum* (Noris & Helena , 2006). The species under consideration is dioecious and forms tubers. Thus the plants are met with in three forms—sterile, male, and female. The sterile plants are once or twice dichotomously divided and the lobes are linear or linear oblong. The appearance of the male plants is variable, the lobes being linear or oblong obcordate. The female plants may be linear or linear-oblong, but more often they are fan-shaped with many growing points on the anterior margin due to rapid dichotomy. The narrow plants are usually less

than 2 mm broad, while the large female plants may be as much as 1 cm long and 2 cm broad in favourable localities. The plants are usually found in dark and moist places forming dense masses of overlapping individuals. In some sterile and smaller male plants, no pores are ever formed. In a large number of them, however, pores are present but on the ventral surface. Each pore is surrounded by two or three series of four or five cells each; these cells are narrow and hyaline and peculiar in that the outer walls of the innermost cells are also concave outwards like the inner walls. The apical parts of the sterile plants and the sterile branches of male plants become transformed into tubers about the end of the rainy season, i.e., about the end of August. The mature tuber is about 1 mm often the tuber is separated from the thallus by a constriction before maturity (Lang, 1905). The biological significance of the pores is that the ventral pores seem to have been produced in order not to reduce the area of the upper surface available for the absorption of the small amount of available light and at the same time to provide for adequate gaseous exchange. The hairs on the upper surface of the tuber protect this region from drought (Goebel, 1905) (Figure 1).

2.1.3. *Pteris vittata*

Pteris vittata L.

Family: Pteridaceae

Common names: Chinese Brake Fern, Brake Fern, Chinese Ladder Fern, ladder break fern.

Pteris vittata is an herbaceous terrestrial or epiphytic fern reported from sea level to elevations of about 2000 m in tropical and subtropical areas (Irving, 1943). It is reported as growing at sides of canals, along streams, banks, road cuttings, forest margins, shaded rock crevices, bases of limestone boulders and exposed areas of pinelands. In the wild, it is found in the lowland tropics in open areas. In urban settings, it often grows in drains or

along old walls. It is a low-growing fern that forms small clumps, rhizome short and scaly. Foliage - dark green fronds are pinnate, with pairs of pinnae (the small leaflet-like structure) lining a single axis. The fronds are odd pinnate, with the terminal pinna longer than the adjacent pinna. Stipe's measuring about 20 cm long, fern blade measuring about 30 - 50 cm long. Sporangia which produce the spores line both edges of each pinna and the sporangia are red with yellow spores (Figure 1).

Uses:

Pteris vittata is being considered for use in the treatment of soils contaminated with arsenic, a highly toxic heavy metal found in some herbicides and insecticides. (Rathinasabapathi, 2006) has suggested using *P. vittata* as a model for the study of arsenic uptake, translocation, speciation, distribution and detoxification in plants. According to (Xie , Li , Guo , & Wang , 2010), the species is a good resource for the observation of meiosis. Extracts from *P. vittata* have shown activity against the gastrointestinal pathogens *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Singh, et al., 2008). *P. vittata* is also able to accumulate cadmium, copper, nickel and zinc (Sarma, 2011). Research is being conducted to explore its potential in phytoremediation, the use of plants to clean polluted environments.

2.1.4. *Adiantum lunulatum*

Adiantum lunulatum Burm.f.

Family: Pteridaceae

Common names: Walking maidenhair fern, or black maidenhair.

Adiantum lunulatum is an evergreen, perennial fern producing fronds up to 40 cm tall from a shortly-creeping rhizome. The fronds are arching to semi-prostrate, forming roots at their

apex from which a new plant develops (Huxley, 1992). *Adiantum lunulatum* has a short rhizome, slightly erect or creeping, wearing dark brown scales of about 3 mm long. The fronds are arched and arranged in tufts. The petiole is black, shiny, hairless, 10 to over 15 cm long. The lamina is lanceolate, pinnate and has a herbaceous texture. The pinnae are alternate, long-stalked, semi-elliptical, dimidiate. Their upper base is truncated. Their top margin is serrated on the sterile frond but slightly lobed on the fertile frond. The terminal pinnae are obtriangular. The sori are crescent shaped. *Adiantum lunulatum* grows almost everywhere, in the shade of trees, on various soils, with sufficient moisture (Abotsi, et al., 2015) (Figure 1).

Uses:

Adiantum lunulatum is an Ayurvedic herb used for the treatment of diarrhea, poisonous effect due to insect bite, wound, herpes, cough, dysuria and hoarseness of voice. The plant is considered to be a bronchio-dilator, diuretic and pectoral. It is used extensively in Indian for the treatment of fevers in children. It is one of the constituents of 'Hansraj', a preparation esteemed in India for the treatment of coughs. The rootstock is considered good for treating fever and elephantiasis. A decoction of the root is used in the treatment of throat affections (Haines, 1922).

2.2. Collection of plant sample

Fresh, healthy plant sample of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) were collected in the month of August from Goa University campus. The specimens were authenticated by Dr. Pradeepesh Bhattacharya. The collected plant samples was thoroughly washed under tap

water and then rinsed with distilled water for the removal of dust and soil particles. The specimens were first shade dried for 24 hour then oven-dried for 48 hour at 50°C and ground to a fine powder using liquid nitrogen. The powdered material was stored at – 20°C until further use.

2.3. Preparation of plant extracts

100% Methanol, ethanol and acetone were used for cold extraction. Cold extract was prepared by taking 10 g powder in 100 mL of solvent (methanol, ethanol and acetone) and kept at room temperature for 72 hour. The extract was stirred after every 4 to 5 hour. The solution was filtered using whatman filter paper and the filtrates were concentrated using rotary evaporator at 40^{o}C. The dried extracts were kept in refrigerator at -4^{o}C, to be used for further study (Blicharski & Oniszczuk, 2017).

2.4. Qualitative Phytochemical Screening

2.4.1. Detection of Alkaloids

Solvent free extracts, 50 mg is stirred with few mL of dilute hydrochloric acid and filtered. The filtrate is tested carefully with various alkaloidal reagents as follows:

A. Mayer's test

To a few mL of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

B. Wagner's test

To a few mL of filtrate, few drops of Wagner's reagent are added by the side of the test tube. A reddish brown precipitate confirms the test as positive.

2.4.2. Detection of Carbohydrates

The extracts (100 mg) is dissolved in 5 mL of water and filtered. The filtrate is subjected to the following test.

A. Benedict's test

To 0.5 mL of filtrate, 0.5 mL of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 min. A characteristic coloured precipitate indicates the presence of sugar.

2.4.3. Detection of Glycosides

50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hour on a water bath, filtered and the hydrolysate is subjected to the following test.

A. Borntrager's test

To 2 mL of filtered hydrolysate, 3 mL of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

2.4.4. Detection of Saponins

The extract (50 mg) is diluted with distilled water and made up to 20 mL. The suspension is shaken in a graduated cylinder for 15 min. A 2 cm layer of foam indicates the presence of saponins.

2.4.5. Detection of Proteins and Amino acids

The extracts (100 mg) is dissolved in 10 mL of distilled water and filtered through whatman No.1 filter paper and the filtrate is subjected to tests for proteins and amino acids.

A. Biuret test

An aliquot of 2 mL of filtrate is treated with one drop of 2% copper sulphate solution. To this, 1mL of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicates the presence of proteins.

B. Ninhydrin test

Two drops of Ninhydrin solution (10 mg of Ninhydrin in 200 mL of acetone) are added to two mL of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

2.4.6. Detection of Fixed oils and Fats

A. Saponification test

A few drops of 0.5 N alcoholic potassium hydroxide solution was added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on water bath for 2

hour. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

2.4.7. Detection of Phenolic compounds

A. Ferric chloride test

The extract (50 mg) is dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds (Raaman, 2006).

2.5. Determination of Total Phenolic Content (TPC)

Total phenolic content was estimated by Folin Ciocalteu's method. Standard Gallic acid solution was prepared by dissolving 10 mg in 10 mL of distilled water (1 mg/ mL). The total phenolics were expressed as mg of Gallic acid equivalents (GAE) /g of extract. Various concentrations of gallic acid solutions in distilled water (0.1, 5, 10, 15, 20 and 25 µg/ mL) were prepared from the standard solution. Concentration of 1mg/ mL of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*) extracts were also prepared in respective solvents (Methanol, Ethanol and Acetone) and 200 µL of each sample were introduced into test tubes and final volume was made to 500 µL using distilled water. Then 150 µL of 10 fold dilute Folin-Ciocalteu reagent was mixed and shaken. After 5 minutes, 2 ml of 7.5% sodium carbonate was added. The test tubes were covered with aluminium foil and incubated for 1 hour at room temperature. After incubation, absorbance was read at 650 nm spectrophotometrically. All determinations were performed in triplicate. The Folin-

Ciocalteu reagent being sensitive to reducing compounds including polyphenols produced a blue colour upon reaction which is measured spectrophotometrically. The calibration curve was plotted using standard gallic acid (Savitree, et al., 2004).

2.6. Antioxidant activity

2.6.1 DPPH radical scavenging assay

Free radical scavenging activity of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*) were determined according to the DPPH methods of (Ahmed et al., 2013) and (Brand-Williams et al., 1995) The hydrogen atom donating ability of the plant extracts was determined by the decolourization of ethanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet / purple colour in ethanol solution and fades to shades of yellow colour in the presence of antioxidants. The stock solution of DPPH radical (24 mg/100 mL in ethanol) was prepared and stored in -20^{o}C until further use. By diluting the stock solution of DPPH with ethanol (10 mL/45 mL ethanol), a working solution was prepared. Stock solutions of plant extracts were prepared in methanol, ethanol and acetone with concentration 3 mg/ mL. From this, different dilutions (12.5-200 μg/ mL) were made. The test mixture contained 3 mL DPPH working solution and 1 mL of a sample. The mixture was incubated at room temperature for 30 min in dark. Absorbance of each sample was recorded at 517 nm. For a negative control, 1 mL of methanol, ethanol and acetone was added in place of plant sample, respectively. Ascorbic acid (10 mg/10 mL distilled water) was used as a positive control. Each extract was analyzed in triplicate. % Inhibition curves were made and IC_{50} values were calculated for all samples. The percentage of inhibition was calculated by using the formula:

$$\% \text{ Inhibition} = \{(A \text{ blank} - A \text{ sample}) / A \text{ blank}\} \times 100$$

Where, *A* blank is the absorbance of the negative control and *A* sample is the absorbance of the sample/standard (Ahmed et al., 2013 and Brand-Williams et al., 1995).

2.7. Antimicrobial activity

2.7.1. Fungal and bacterial species

The fungal (*Aspergillus* sp.) strain was obtained from Goa University fungal culture collection lab (GUFCC), Department of Botany, Goa University and was maintained in malt-extract agar (MEA) slant at room temperature. The bacterial (*Escherichia coli*) strain was obtained from Mr. Anchit Parkar, Department of Microbiology, PES's College of Arts & Science, Farmagudi and was maintained in nutrient agar slant at 4^{o}C for experimental studies.

2.7.2. Preparation of sterile disc

Whatman's No.1 filter paper was punched into 6 mm disc form and then sterilized by autoclaving; each sterile disc was individually soaked in extracts for 5 min and then was allowed to air dry.

2.7.3. Assay for antimicrobial activity using disc diffusion method

Sterilized Potato Dextrose Agar (PDA) (250 mL) and nutrient agar were poured into sterile petriplates, after solidification, 100 µL of fresh fungal (*Aspergillus* sp.) and bacterial (*Escherichia coli*) inoculum was spread plate onto their respective plates. The dried discs were placed in the centre of the agar plates using sterile forceps. The plates were incubated for 48 hours at room temperature. After incubation, the diameter of inhibitory zones formed around each discs were recorded (Mahalngam et al., 2011).

2.7.4. Antimicrobial activity of commercially available antibiotics (Positive and negative control)

The antimicrobial activity of the plant extracts on fungal (*Aspergillus* sp.) strain and bacterial (*Escherichia coli*) strain were compared with the commercially available antibiotics. Ketoconazole fungal positive control and gentamicin bacterial positive control (10 mg/ mL) were used. Sterile Potato Dextrose Agar (PDA) and nutrient Agar plates were prepared and the test organisms were spread plate onto their respective agar plates. The antibiotic discs (Ketoconazole and gentamicin) were placed in the centre of the agar plates using sterile forceps. The plates were incubated for 48 hours at room temperature and after incubation the diameter of the inhibition zones were recorded. 100% methanol, ethanol and acetone were used as negative control (Daniyan & Mahammad, 2008).

2.8. Thin Layer Chromatography and UV-VIS spectra

2.8.1. TLC of Phenolic acids

Pre-coated Merck TLC Silica Aluminium Plates measuring 20 x 20 cm were used. For this experiment, four mobile phase solvent systems were used;

Solvent system I – Chloroform: Ethyl acetate: Formic acid (5:4:1),

Solvent system II – Toluene: Acetone: Formic acid (4.5:4.5:1),

Solvent system III – Ethyl acetate: Methanol: Water (100:13.5:10) and

Solvent system IV – Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26).

The methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*) (20 μL) were loaded as concentrated bands, 1.5 – 2 cm from the edge of its respective TLC plate and allowed to dry. The plates, with dried samples, were gently lowered into the chromatography chamber, closed and left to develop. The plates were removed from the chromatography chamber when the solvent front had travelled $3/4^{th}$ of the plate's length. The position of the solvent front was immediately marked with a soft pencil. Phenolic acids were detected using ultraviolet trans-illuminator (Long UV = 365 nm). For a better resolution of the separated phenolic acids and for photography records, the chromatogram was then exposed to ammonia fumes for 10 minutes. The retention factor (R_f) values of the different bands were then calculated using the equation (Wagner & Bladt, 1996).

2.8.2. TLC of Flavonoids

Pre-coated Merck TLC Silica Aluminium plates measuring 20 x 20 cm were used. For this investigation, four mobile phase solvent systems were used;

Solvent system I – Ethyl acetate: Acetic acid: Formic acid: Water (100:11:11:26),

Solvent system II – Methanol: Chloroform: Hexane (7:2:1),

Solvent system III – Butanol: Acetic acid: Water (6:1:2) and

Solvent system IV – Toluene: Ethyl acetate: Glacial acetic acid (30:40:5).

The methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*) (20 μL) were loaded as concentrated spot, 1.5 – 2 cm from the edge of its respective TLC plate and allowed to dry. The plates with dried samples, were gently lowered into the chromatography chamber, closed and left to develop. The plates were removed from the chromatography chamber when the solvent front had travelled $3/4^{th}$ of the plate's length. The position of the solvent front was immediately marked with a soft pencil. Flavonoids were detected using ultraviolet trans-illuminator (Long UV = 365nm). For a better resolution of the separated flavonoids and for photography records, the chromatogram was then exposed to ammonia fumes for 10 minutes. The retention factor (R_f) values of the different bands were then calculated using the equation (Wagner & Bladt, 1996).

2.8.3. TLC of Plant pigments

Pre-coated Merck TLC Silica Aluminium plates measuring 20 x 20 cm was used. For this investigation, solvent system – Petroleum ether: Acetone: Chloroform (3:1:1) was used.

The acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*) (20 µL) were loaded as concentrated bands, 1.5 – 2 cm from the edge of its respective TLC plate and allowed to dry. The plates with dried samples, were gently lowered into the chromatography chamber, closed and allowed to develop. The plates were removed from the chromatography chamber when the solvent front had travelled $3/4^{th}$ of the plate's length. The position of the solvent front was immediately marked with a soft pencil. The retention factor (R_f) values of the different bands were then calculated using the equation (Wagner & Bladt, 1996).

2.8.4. UV-VIS spectra

The methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*) were examined under UV and visible light for proximate analysis, using UV – VIS Spectrophotometer. The extracts were diluted to 1:9 with the respective solvent and scanned in the wavelength ranging from 190 - 700 nm. The characteristic peaks were detected.

3. RESULTS

3.1. Phytochemical tests

The qualitative analysis of phytochemical constituents present in methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) are shown in Table No. 1, 2, 3 and Figure No. 2, 3, 4, 5, 6, 7.

The phytochemicals present in methanolic extract of Bryophytes and Pteridophytes are given in table No. 1 and Figure No. 2, 3. *In Funaria hygrometrica and Cyathodium tuberosum*, carbohydrates and proteins were present, while in *Pteris vittata*, two more phytochemicals were observed i.e., glycosides and saponins in addition to carbohydrates and proteins. In *Adiantum lunulatum*, apart from carbohydrate and proteins, fixed oils and fats were present.

The phytochemicals present in ethanolic extract of Bryophytes and Pteridophytes are given in table No. 2 and Figure No. 4, 5. Carbohydrates and proteins were found in all species studied. In *Pteris vittata* and *Adiantum lunulatum*, more phytochemicals like saponins, fixed oils and fats were also present.

The phytochemicals present in acetone extract of Bryophytes and Pteridophytes are shown in table No. 3 and Figure No. 6, 7. *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum* showed presence of carbohydrates, proteins and fixed oils and fats. In *Pteris vittata* and *Adiantum lunulatum*, saponins and phenolic compounds were also found.

Overall, very few phytochemicals were observed in methanolic, ethanolic and acetone extracts of Bryophytes as compared to Pteridophytes.

3.2. Quantitative estimation of Total Phenolic Content (TPC)

The results of total phenolic content in methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) are shown in Table No. 4 and Figure No. 8, 9.

The methanolic extract of *Pteris vittata* (37.20 %) showed higher total phenolic content (TPC) as compared to the methanolic extracts of *Funaria hygrometrica* (10.24 %), *Cyathodium tuberosum* (23.53 %) and *Adiantum lunulatum* (29.03%) (Table No. 4 and Figure No. 9).

The ethanolic extract of *Pteris vittata* (37.25 %) also showed higher total phenolic content (TPC) as compared to the other ethanolic extracts of *Funaria hygrometrica* (7.230 %), *Cyathodium tuberosum* (24.98 %) and *Adiantum lunulatum* (30.54 %) (Table No. 4 and Figure No. 9).

The acetone extract of *Adiantum lunulatum* (41.09 %) showed higher total phenolic content (TPC) as compared to the acetone extracts of *Funaria hygrometrica* (9.328 %), *Cyathodium tuberosum* (19.98 %) and *Pteris vittata* (29.61 %) (Table No. 4 and Figure No. 9).

Overall, Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) showed a higher total phenolic content (TPC) as compared to Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*).

3.3. Antioxidant activity

Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. DPPH scavenging activity of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) are shown in Table No. 5, 6, 7, 8 and Figure No. 10, 11, 12, 13.

The results of percent (%) scavenging activity of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) are compared with the standard L - Ascorbic acid. It was observed that % Scavenging activity increases with increase in concentration of extract.

Table No. 8 and Figure No. 11 showed high percent (%) scavenging activity in the methanolic extracts of Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) as compared to Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*). The percent scavenging activity of *Pteris vittata* was higher than that of *Adiantum lunulatum.*

The ethanolic extracts of Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) showed high % scavenging activity as compared to Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*). Here % scavenging activity of *Pteris vittata* was higher than that of *Adiantum lunulatum* (Table No. 8 and Figure No. 12).

The acetone extracts of Pteridophytes showed high % Scavenging activity as compared to Bryophytes. *Adiantum lunulatum* showed higher % Scavenging activity than that of *Pteris vittata* (Table No. 8 and Figure No. 13).

Lower the IC_{50} value, higher the antioxidant activity. It was observed that the methanolic extract of *Pteris vittata* showed a higher antioxidant activity (IC_{50} = 110.91 µg/ mL) (Table No. 8). The ethanolic extract of *Pteris vittata* also showed a higher antioxidant activity (IC_{50} = 1166.11 µg/mL) (Table No. 8). The acetone extract of *Adiantum lunulatum* showed a higher antioxidant activity (IC_{50} = 408.45 µg/ mL) (Table No. 8).

Overall, the methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) revealed low antioxidant activity as compared to the standard, L - Ascorbic acid.

3.4. Antimicrobial activity

The antifungal activity of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) on fungal (*Aspergillus* sp.) strain are shown in Table No. 9 and Figure No. 14, 15, 16. The methanolic, ethanolic and acetone extracts of Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) showed more inhibition of growth of the fungal (*Aspergillus* sp.) strain as compared to Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum).* The methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) showed less inhibition of growth of the fungal (*Aspergillus* sp.) strain as compared to *Pteris vittata*, *Adiantum lunulatum* and positive control (Ketoconazole).

The antibacterial activity of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) on bacterial (*Escherichia coli*) strain are shown in Table No. 10 and Figure No. 17, 18, 19. The methanolic and acetone extracts of *Pteris vittata* showed less inhibition of growth of the bacterial (*Escherichia coli*) strain as compared to its ethanolic extract, which showed no inhibition. The methanolic and ethanolic extracts of *Adiantum lunulatum* showed less inhibition of growth of the bacterial (*Escherichia coli*) strain as compared to its acetone extract, which showed no inhibition. The methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) showed no inhibition of growth of the bacterial (*Escherichia coli*) strain as compared to the Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) and positive control (Gentamicin).

3.5. Thin Layer Chromatography (TLC) and UV-VIS spectra

Thin layer chromatography (TLC) and UV-VIS spectra was carried out for separation of phenolic acids, flavonoids and plant pigments in methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) (Table No. 11, 12, 13, 14, 15, 16, 17 and Figure No. 20, 21, 22, 23, 24, 25). Phenolic acids, Flavonoids and Plant pigments were identified based on the standard retention factor (Rf) values and UV-VIS peak values.

3.5.1. Separation of phenolic acids:

Thin layer chromatography (TLC) separation of Phenolic acids was carried out using four mobile phase solvent systems to determine which solvent system can separate Phenolic acids accurately. It was observed that Phenolic compounds were separated using Solvent system II – Toluene: Acetone: Formic acid (4.5:4.5:1) after exposure to ammonia fumes (Figure No. 20).

The Phenolic compounds tentatively identified in methanolic extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum* included Salicylic (Rf - 0.88, 0.93), Protocatechuic (Rf – 0.12, 0.15), 4-Methylresorcinol (Rf – 0.23), 2- Methylresorcinol (Rf – 0.43), p-Hydroxybenzoic (Rf – 0.49), Syringic (Rf – 0.68, 0.79) and Vanillic (Rf – 0.86, 0.87). The Rf values, λ max, colour of the bands and UV-VIS spectra are shown in Table No. 11 and Figure No. 20, 23. The phenolic compounds tentatively identified in ethanolic extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum* included Syringic (Rf – 0.77, 0.73, 0.76), Vanillic (Rf – 0.84, 0.85, 0.87), Resorcinol (Rf – 0.14), 4-Methylresorcinol (Rf –

0.21), Gentisic (Rf – 0.33, 0.34), p-Hydroxybenzoic (Rf – 0.59) and 2- Methylresorcinol (Rf – 0.42). The Rf values, λ max, colour of the bands and UV-VIS spectra are shown in Table No. 12 and Figure No. 20, 24. The phenolic compounds tentatively identified in acetone extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum* included Salicylic (Rf – 0.92, 0.93, 0.95, 0.94), Pyrogallol (Rf – 0.09, 0.10), Catechol (Rf – 0.34, 0.36), 2- Methylresorcinol (0.44, 0.45), p-Hydroxybenzoic (Rf – 0.52), Syringic (Rf – 0.72, 0.78), Vanillic (Rf – 0.87) and 4-Methylresorcinol (Rf – 0.25). The Rf values, λ max, colour of the bands and UV-VIS spectra are shown in Table No. 13 and Figure No. 20, 25.

3.5.2. Separation of flavonoids:

Thin layer chromatography (TLC) separation of Flavonoids was carried out using four mobile phase solvent systems to determine which solvent system can separate Flavonoids accurately. It was observed that Flavonoids were separated using Solvent system II – Methanol: Chloroform: Hexane (7:2:1) after exposure to ammonia fumes (Figure 21).

The flavonoid compounds tentatively identified in methanolic extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum* included Luteolin (Rf – 0.79), Kaempferol (Rf – 0.84), Kayaflavone (Rf – 0.93, 0.95), Orientin (Rf – 0.33), Azaleatin (Rf – 0.47, 0.48), Quercetin (0.65, 0.66), Isorhamnetin (Rf – 0.74), Apigenin (Rf – 0.86) and Isovitexin (Rf – 0.57). The Rf values, λ max, colour of the bands and UV-VIS spectra are shown in Table No. 14 and Figure No. 21, 23. The flavonoid compounds tentatively identified in ethanolic extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum* included Luteolin (Rf – 0.77, 0.78), Kaempferol (Rf – 0.84, 0.85), Apigenin (Rf – 0.89), Orientin (Rf – 0.29, 0.30),

Isorhamnetin (Rf – 0.75), Azaleatin (Rf – 0.47), Isovitexin (Rf – 0.51) and Quercetin (Rf – 0.66). The Rf values, λ max, colour of the bands and UV-VIS spectra are shown in Table No. 15 and Figure No. 21, 24. The flavonoid compounds tentatively identified in acetone extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum* included Chrysoeriol (Rf – 0.80, 0.81, 0.82), Apigenin (Rf – 0.86, 0.89, 0.90), Kayaflavone (Rf – 0.93, 0.96, 0.97, 0.98), Isorhamnetin (Rf – 0.74), Kaempferol (Rf – 0.85), Azaleatin (Rf – 0.49), Isovitexin (Rf – 0.54), Quercetin (Rf – 0.64,0.69) and Luteolin (Rf –0.77, 0.76). The Rf values, λ max, colour of the bands and UV-VIS spectra are shown in Table No. 16 and Figure No. 21, 25.

3.5.3. Separation of photosynthetic pigments:

The photosynthetic pigments were identified using thin layer chromatography (TLC). Photosynthetic pigments such as Chlorophyll a, Chlorophyll b, Chlorophyll c, β-carotene, Phaeophytin a, Lutein, Violaxanthin, Neoxanthin and Fucoxanthin were observed in Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) (Table No. 17 and Figure No. 22).

In *Funaria hygrometrica*, six photosynthetic pigments tentatively identified are Chlorophyll a (Rf - 0.68), Chlorophyll b (Rf - 0.54), β-carotene (Rf - 0.94), Phaeophytin a (Rf - 0.85), Fucoxanthin (Rf - 0.51) and Violaxanthin (Rf - 0.22), showing colour blue-green, yellow-green, yellow/orange, grey, orange brown and yellow respectively (Table No. 17 and Figure No. 22).

In *Cyathodium tuberosum*, six photosynthetic pigments tentatively identified are Chlorophyll a (Rf - 0.68), Chlorophyll b (Rf - 0.54), β-carotene (Rf - 0.94), Phaeophytin a (Rf - 0.85), Fucoxanthin (Rf - 0.51) and Violaxanthin (Rf - 0.22), showing colour blue-

green, yellow-green, yellow/orange, grey, orange brown and yellow respectively (Table No. 17 and Figure No. 22).

In *Pteris vittata*, seven photosynthetic pigments tentatively identified are Chlorophyll a (Rf - 0.68), Chlorophyll b (Rf - 0.54), Chlorophyll c (Rf - 0.03), β-carotene (Rf - 0.94), Phaeophytin a (Rf - 0.85), Fucoxanthin (Rf - 0.51) and Violaxanthin (Rf - 0.22), showing colour blue-green, yellow-green, light green, yellow/orange, grey, orange brown and yellow respectively (Table No. 17 and Figure No. 22).

In *Adiantum lunulatum*, eight photosynthetic pigments tentatively identified are Chlorophyll a (Rf - 0.68), Chlorophyll b (Rf - 0.54), Chlorophyll c (Rf - 0.03), β-carotene (Rf - 0.94), Phaeophytin a (Rf - 0.85), Lutein (Rf – 0.43), Violaxanthin (Rf - 0.22) and Neoxanthin (Rf – 0.08), showing colour blue-green, yellow-green, light green, yellow/orange, grey, yellow, yellow and yellow respectively (Table No. 17 and Figure No. 22).

4. DISCUSSION

Our results on phytochemical screening reveals that the phytochemical compounds present in Bryophytes include carbohydrates and proteins where as in Pteridophytes it includes carbohydrates, glycosides, saponins, proteins, fixed oils and fats and phenolic compounds (Table No. 1, 2, 3 and Figure No. 2, 3, 4, 5, 6, 7). Deora (2015) has observed that selected bryophytes showed the presence of terpenoids, flavonoids, steroids, and glycosides were present 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. Phytochemical studies on the genus *Pteris* (Pteridaceae) have yielded a variety of secondary metabolites including ent-kaurane diterpenoids and pterosin-sesquiterpenes (Wang et al. 2011), flavonoids, benzenoids and benzenoid derivatives (Chen et al. 2007). Pterosins and ent-kaurane diterpenoids are the characteristic constituents of the fern family Pteridaceae.

Phenolic compounds contained in plants have redox properties, and these properties allow them to act as antioxidants (Shoib & Shahid, 2015). The results showed that the methanolic extract of *Pteris vittata*, ethanolic extract of *Pteris vittata* and acetone extract of *Adiantum lunulatum* showed higher total phenolic content (TPC) as compared to Bryophytes (Table No. 4 and Figure No. 8, 9). A linear correlation between antioxidant activity and the content of polyphenols have been reported by Katalinic et al. (2006). A positive correlation coefficient between the total phenolic content and DPPH assay of plants extracts obtained from kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) was reported by Maizura et al. (2011).

The results of antioxidant activity correlated with the results of total phenolic content (TPC). It was observed that the methanolic and ethanolic extract of *Pteris vittata*

and acetone extract of *Adiantum lunulatum* showed a higher antioxidant activity as compared to Bryophytes (Table No. 5, 6, 7, 8 and Figure No. 10, 11, 12, 13). Lai and Lim (2011) reported that *Pteris vittata, Cyathea latebrosa, Cibotium barometz*, D. *quercifolia, B. orientale, Adiantum radianum, Diplazium esculentum, Acrostichum aureum, Nephrolepis biserrata,* and *D. linearis* exhibited strong antioxidant activity ranging from 0.12 to 0.57 mg/ml. The presence of phenolics in extracts of *Dicksonia sellowiana* may contribute directly to its antioxidant properties (Bora et al., 2005). Methanol leaf extracts of *Pteris multifida* showed stronger antioxidant activity than ethanol extracts (Hoang and Tran, 2014). The antioxidant activity has been reported for several fern species, namely *Polystichum semifertile*, *Nothoperanema hendersonii*, *Braomea insignis* (Chen K et al., 2005) and *Selaginella* sp. (Gayathri et al., 2005).

Our results of antifungal activity of methanolic, ethanolic and acetone extracts of Pteridophytes showed more inhibition of growth of the fungal (*Aspergillus* sp.) strain as compared to Bryophytes (Table No. 9 and Figure No. 14, 15, 16). Bryophytes have been reported to have antifungal activity (Banerjee and Sen, 1979) and have shown antibiosis against a number of plant pathogenic fungi. Singh, (1999) and reported that fungicidal activity might be due to the presence of phenolic compounds present in the ferns. Compounds isolated from bryophytes have shown reversal of conventional antibiotic resistance developed in human pathogenic fungi (Xie and Lou, 2008). The problem of drug resistance development in pathogenic fungi (Vanden Bossche et al., 1998) can be solved by using novel biomolecules derived from unique natural sources like bryophytes. The antimycotic properties of pteridophytes are widely reported by Sen and Nandi (1951).

Our results of antibacterial activity of methanolic, ethanolic and acetone extracts of Pteridophytes showed less inhibition of growth of the bacterial (*Escherichia coli*) strain (Table No. 10 and Figure No. 17, 18, 19). The production of antibacterial substances is a

wide-spread phenomenon in bryophytes (Asakawa, 1990). Bryophytes inhibits the growth of maximum pathogens which might be due to the presence of maximum phytoconstituents. Antimicrobial activity has been reported in the extracts of many liverworts and the existence of high antibacterial activity in case of liverworts may be due to the presence of biologically active compounds in them. Our results showed very little microbial activity in pteridophytes, which was supported by Dhar et al., (1968). They also did not find any antimicrobial activity in the 42 species of pteridophytes examined by them.

The Phenolic compounds tentatively identified in methanolic, ethanolic and acetone extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum* using TLC include Salicylic, Protocatechuic, 4-Methylresorcinol, 2-Methylresorcinol, p-Hydroxybenzoic, Resorcinol, Gentisic, Pyrogallol, Catechol, Syringic and Vanillic. The Flavonoid compounds tentatively identified in methanolic ethanolic and acetone extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum* using TLC include Luteolin, Kaempferol, Kayaflavone, Orientin, Azaleatin, Quercetin, Isorhamnetin, Apigenin, Quercetin, Chrysoeriol and Isovitexin. The photosynthetic pigments tentatively identified in Bryophytes and Pteridophytes using TLC are Chlorophyll a, Chlorophyll b, Chlorophyll c, β-carotene, Phaeophytin a, Lutein, Violaxanthin, Neoxanthin and Fucoxanthin (Table No. 11, 12, 13, 14, 15, 16, 17 and Figure No. 20, 21, 22, 23, 24, 25). Sawant and Karadge (2010) suggested that the extracts of three liverworts, *Plagiochasma intermedium*, *Asterella wallichiana* and *Targionia hypophylla* in methanol and dichloromethane solvents have a broad spectrum of activity although the degree of susceptibility could differ between different organisms.

Lee and Lin, (1988) has worked on another species of *Pteris multifida,* which has various bioactive flavonoids with heat-clearing, antipyretic, detoxification, antibiotic, anti-inflammatory, and antimutagenic activity. They reported that entire plant of *P. multifida* contains various bioactive compounds such as terpenoids, especially the sesquiterpenes which form a significant group of secondary metabolites and have a varied range of medicinal activity, including anti-cancer, anti-inflammatory, cytotoxic, plant growth-regulatory, and antimicrobial properties (Baruah et al., 1994).

Bryophytes and pteridophytes are traditionally used in India by different cultural groups for burns, cuts, wounds, and skin diseases, suggesting that they protect the skin and open wounds from microbial pathogens (Saxena & Harinder, 2004). Some reports are available for the chemical constituents of these bryophytes such as sesquiterpenoids and cyclic bis-bibenzyls (Asakawa, 1999); sesquiterpene lactones, lunularic acid, and flavonoids (apigenin, luteolin, etc.) and apigenin, apigenin-7-O-triglycoside, vitexin and luteolin-7-O-neohesperidoside, and saponarin (Basile et al., 1999).

The biological activities of liverworts are due to terpenoids, aromatic compounds and acetogenins which are present in oil bodies of each species. Most compounds found in the liverworts are composed of lipophilic mono-, sesqui- and di-terpenoids and aromatic compounds. Most sesqui- and di-terpenoids obtained from liverworts are enantiomers of those found in higher plants. Many of these compounds display a characteristic odor, and can have interesting biological activities (Yoshinori et al., 2013). Ferns produce a wide array of secondary metabolites endowed with different bioactivities that could potentially be useful in the treatment of many diseases. Phytochemical studies on ferns have revealed that they contain a wide range of alkaloids, flavonoids, polyphenols, terpenoids, and steroids. The structures of these compounds usually differ from those of related secondary metabolites produced by other higher plants, making them a potentially valuable source of

chemical diversity (Cao et al., 2017). A literature search revealed very little studies on the antimicrobial activity of Indian bryophytes and pteridophytes from this region. Hence further research is recommended for the isolation of bioactive compounds as well as screening of various bioactive properties such as antitumor, antibiotics, etc. of the potent lower plants.

5. CONCLUSION

The result of phytochemical screening reveals that the phytochemical compounds present in Bryophytes include carbohydrates and proteins whereas in Pteridophytes it includes carbohydrates, glycosides, saponins, proteins, fixed oils and fats and phenolic compounds. Overall, very few phytochemicals were observed in methanolic, ethanolic and acetone extracts of Bryophytes as compared to Pteridophytes. The amount of Total Phenolic Content (TPC) in methanolic and ethanolic extract of *Pteris vittata* and acetone extract of *Adiantum lunulatum* was higher as compared to Bryophytes. The results of antioxidant activity correlated with the results of total phenolic content (TPC). The methanolic and ethanolic extract of *Pteris vittata* and acetone extract of *Adiantum lunulatum* showed a higher antioxidant activity as compared to Bryophytes. The antifungal activity of methanolic, ethanolic and acetone extracts of Pteridophytes showed more inhibition of growth of the fungal (*Aspergillus* sp.) strain as compared to Bryophytes whereas the antibacterial activity of methanolic, ethanolic and acetone extracts of Pteridophytes showed less inhibition of growth of the bacterial (*Escherichia coli*) strain. The Phenolic compounds tentatively identified in methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) using TLC include Salicylic, Protocatechuic, 4-Methylresorcinol, 2- Methylresorcinol, p-Hydroxybenzoic, Resorcinol, Gentisic, Pyrogallol, Catechol, Syringic and Vanillic whereas the Flavonoid compounds tentatively identified by TLC include Luteolin, Kaempferol, Kayaflavone, Orientin, Azaleatin, Quercetin, Isorhamnetin, Apigenin, Quercetin, Chrysoeriol and Isovitexin. The photosynthetic pigments tentatively identified in Bryophytes and Pteridophytes using TLC are Chlorophyll a, Chlorophyll b, Chlorophyll c, β-carotene, Phaeophytin a, Lutein, Violaxanthin, Neoxanthin and Fucoxanthin. Overall, the study indicates that the

methanolic, ethanolic and acetone extracts of Pteridophytes showed better results as compared to Bryophytes.

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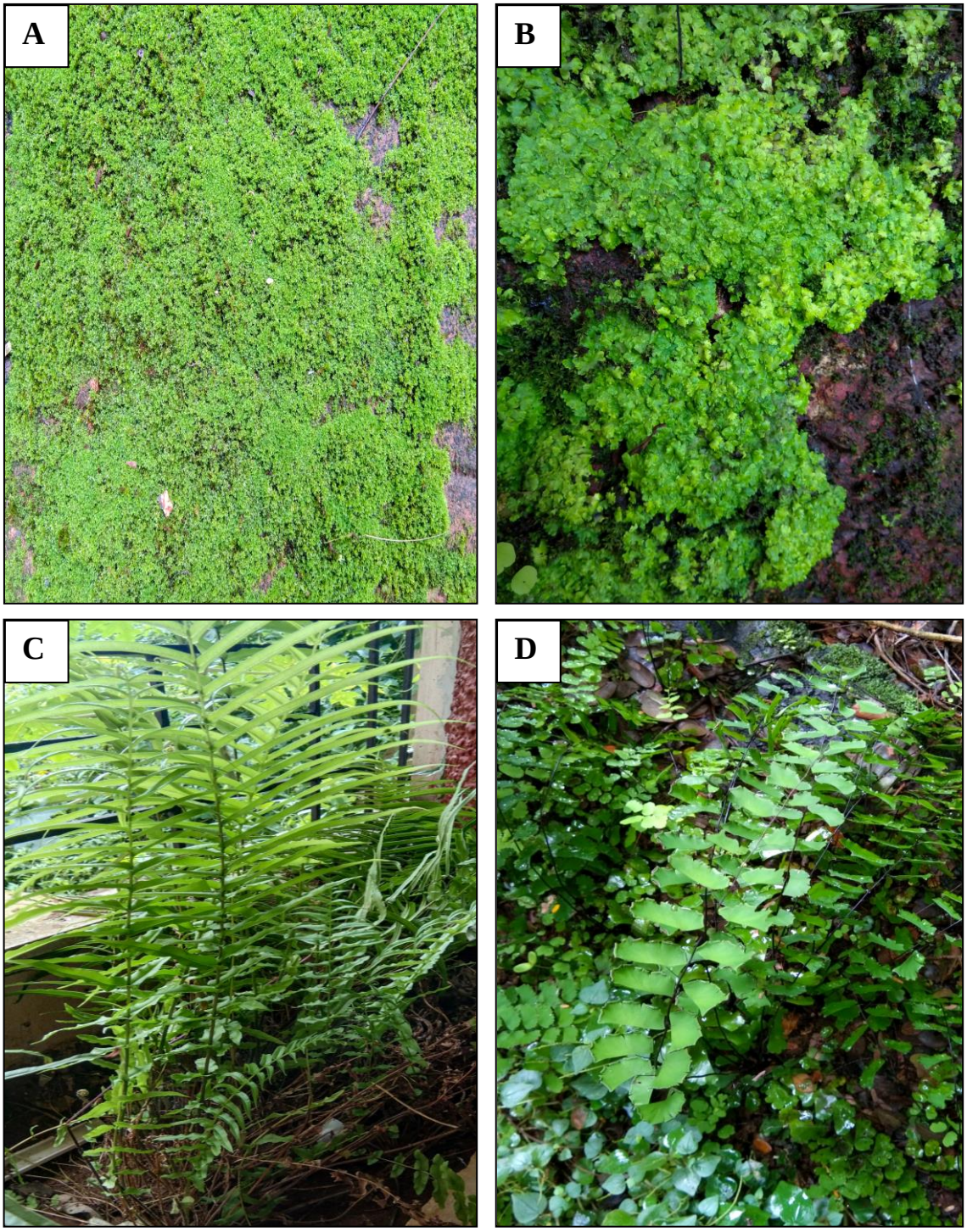


Figure 1: Habit of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata*, *Adiantum lunulatum*. (A) *Funaria hygrometrica*; (B) *Cyathodium tuberosum*; (C) *Pteris vittata*; (D) *Adiantum lunulatum*

Table No. 1: Qualitative analysis of phytochemical constituents present in methanolic extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*).

Sr.No.	Phytochemical test	Methanolic extracts			
		Funaria hygrometrica	*Cyathodium tuberosum*	*Pteris vittata*	*Adiantum lunulatum*
1.	**Detection of Alkaloids** a) Mayer's test b) Wagner's test	- -	- -	- -	- -
2.	**Detection of Carbohydrates** a) Benedict's test	+	+	+	+
3.	**Detection of Glycosides** a) Borntrager's test	-	-	+	-
4.	**Detection of Saponins**	-	-	+	-
5.	**Detection of Proteins and Amino acids** a) Biuret test b) Ninhydrin test	+ -	+ -	+ -	+ -
6.	**Detection of Fixed oils and Fats** a) Saponification test	-	-	-	+
7.	**Detection of Phenolic compounds** a) Ferric chloride test	-	-	-	-

- Negative; + Positive

Table No. 2: Qualitative analysis of phytochemical constituents present in ethanolic extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr.No.	Phytochemical test	Ethanolic extracts			
		Funaria hygrometrica	*Cyathodium tuberosum*	*Pteris vittata*	*Adiantum lunulatum*
1.	**Detection of Alkaloids** a) Mayer's test b) Wagner's test	- -	- -	- -	- -
2.	**Detection of Carbohydrates** a) Benedict's test	+	+	+	+
3.	**Detection of Glycosides** a) Borntrager's test	-	-	-	-
4.	**Detection of Saponins**	-	-	+	+
5.	**Detection of Proteins and Amino acids** a) Biuret test b) Ninhydrin test	+ -	+ -	+ -	+ -
6.	**Detection of Fixed oils and Fats** a) Saponification test	-	-	+	+
7.	**Detection of Phenolic compounds** a) Ferric chloride test	-	-	-	-

- Negative; + Positive

Table No. 3: Qualitative analysis of phytochemical constituents present in acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr.No.	Phytochemical test	Acetone extracts			
		Funaria hygrometrica	*Cyathodium tuberosum*	*Pteris vittata*	*Adiantum lunulatum*
1.	**Detection of Alkaloids** a) Mayer's test b) Wagner's test	- -	- -	- -	- -
2.	**Detection of Carbohydrates** a) Benedict's test	+	+	+	+
3.	**Detection of Glycosides** a) Borntrager's test	-	-	-	-
4.	**Detection of Saponins**	-	-	+	+
5.	**Detection of Proteins and Amino acids** a) Biuret test b) Ninhydrin test	+ -	+ -	+ -	+ -
6.	**Detection of Fixed oils and Fats** a) Saponification test	-	+	+	+
7.	**Detection of Phenolic compounds** a) Ferric chloride test	-	-	-	+

- Negative; + Positive

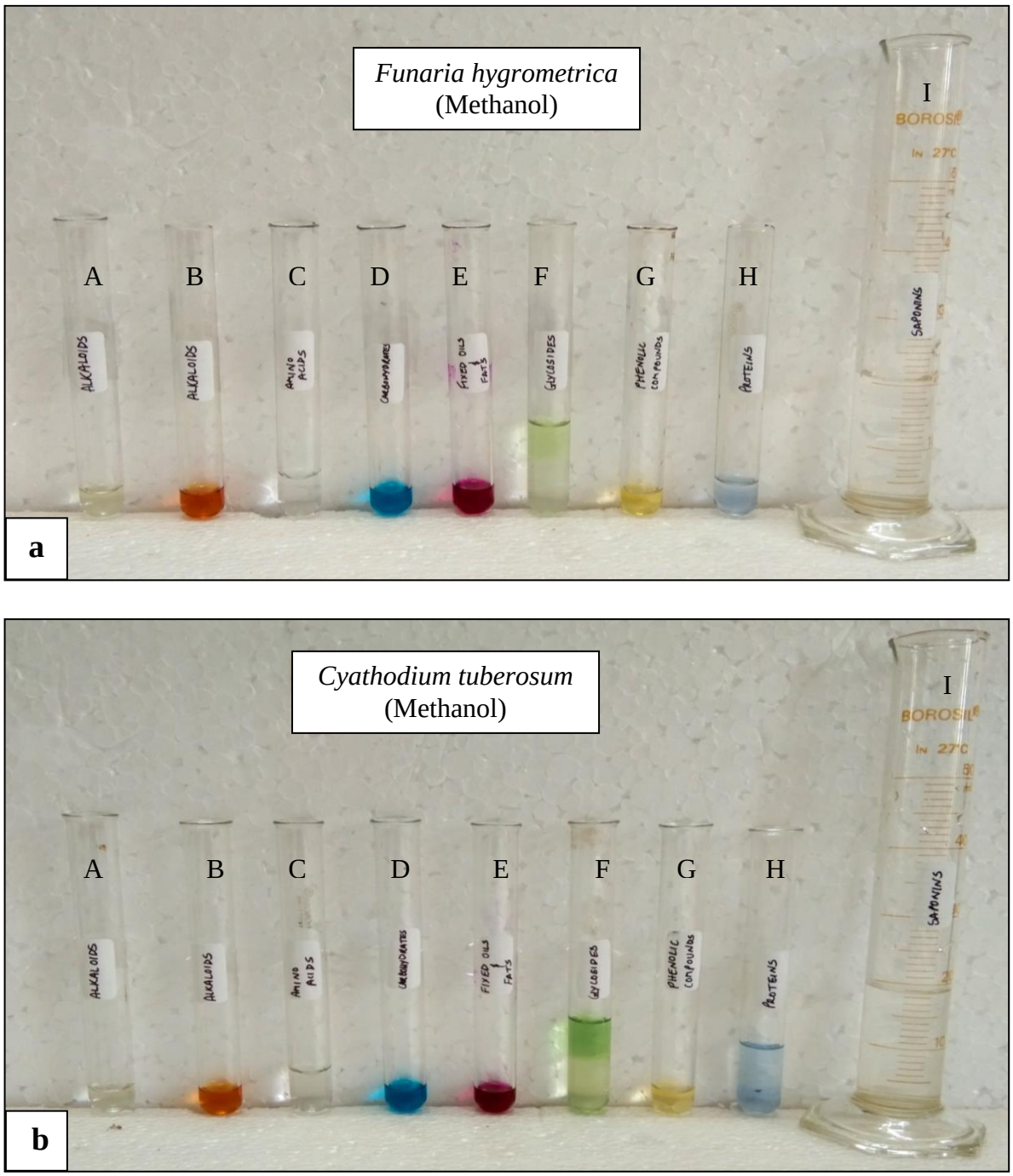


Figure 2: Preliminary phytochemical analysis of methanolic extracts of Bryophytes (a) *Funaria hygrometrica* and (b) *Cyathodium tuberosum*. A. Mayer's test for detection of Alkaloids; B. Wagner's test for detection of Alkaloids; C. Ninhydrin test for detection of Amino acids; D. Benedict's test for detection of Carbohydrates; E. Saponification test for detection of Fixed oils and Fats; F. Borntrager's test for detection of Glycosides; G. Ferric chloride test for detection of Phenolic compounds; H. Biuret test for detection of Proteins; I. Detection of Saponins.

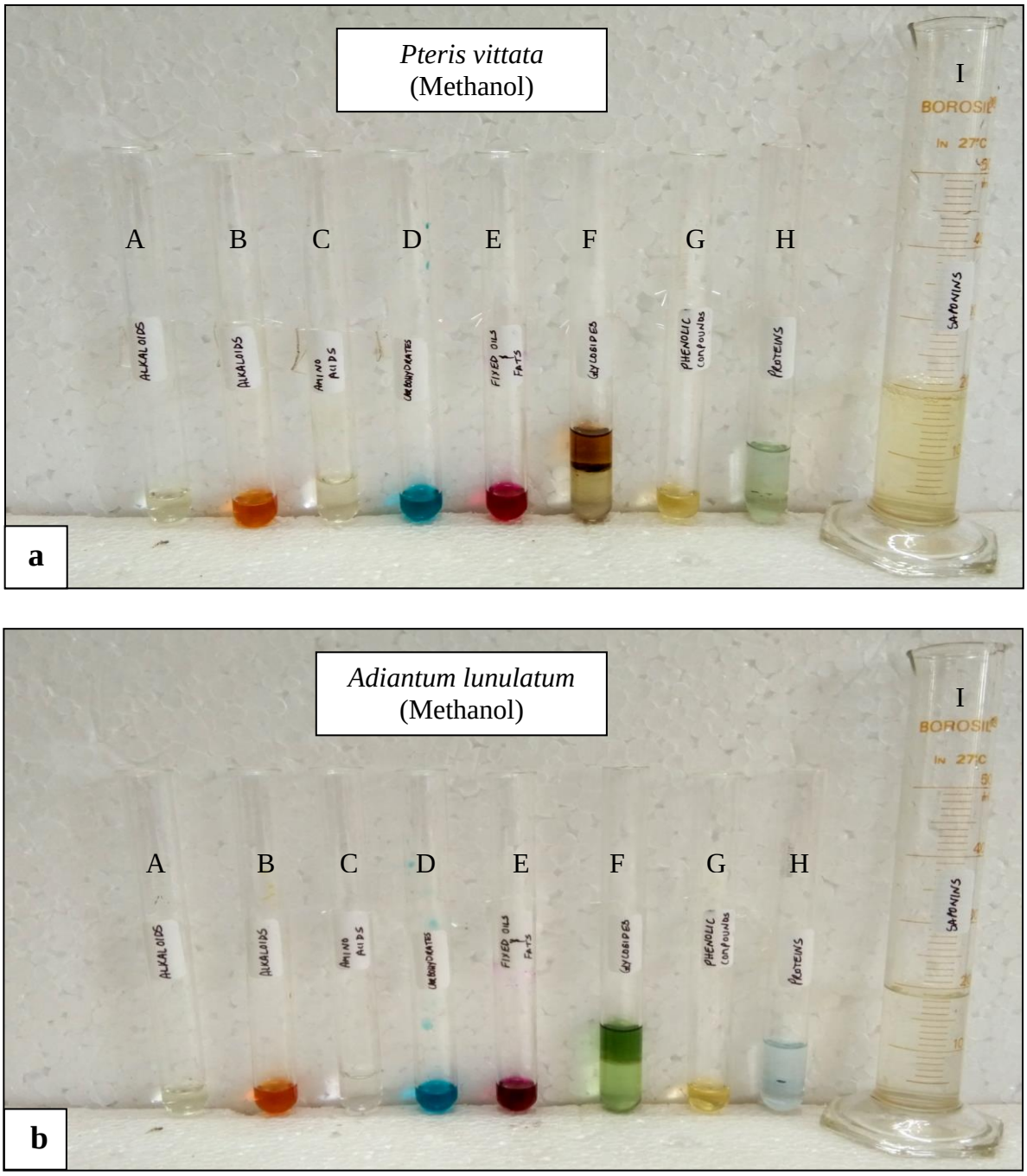


Figure 3: Preliminary phytochemical analysis of methanolic extracts of Pteridophytes (a) *Pteris vittata* and (b) *Adiantum lunulatum*. A. Mayer's test for detection of Alkaloids; B. Wagner's test for detection of Alkaloids; C. Ninhydrin test for detection of Amino acids; D. Benedict's test for detection of Carbohydrates; E. Saponification test for detection of Fixed oils and Fats; F. Borntrager's test for detection of Glycosides; G. Ferric chloride test for detection of Phenolic compounds; H. Biuret test for detection of Proteins; I. Detection of Saponins.

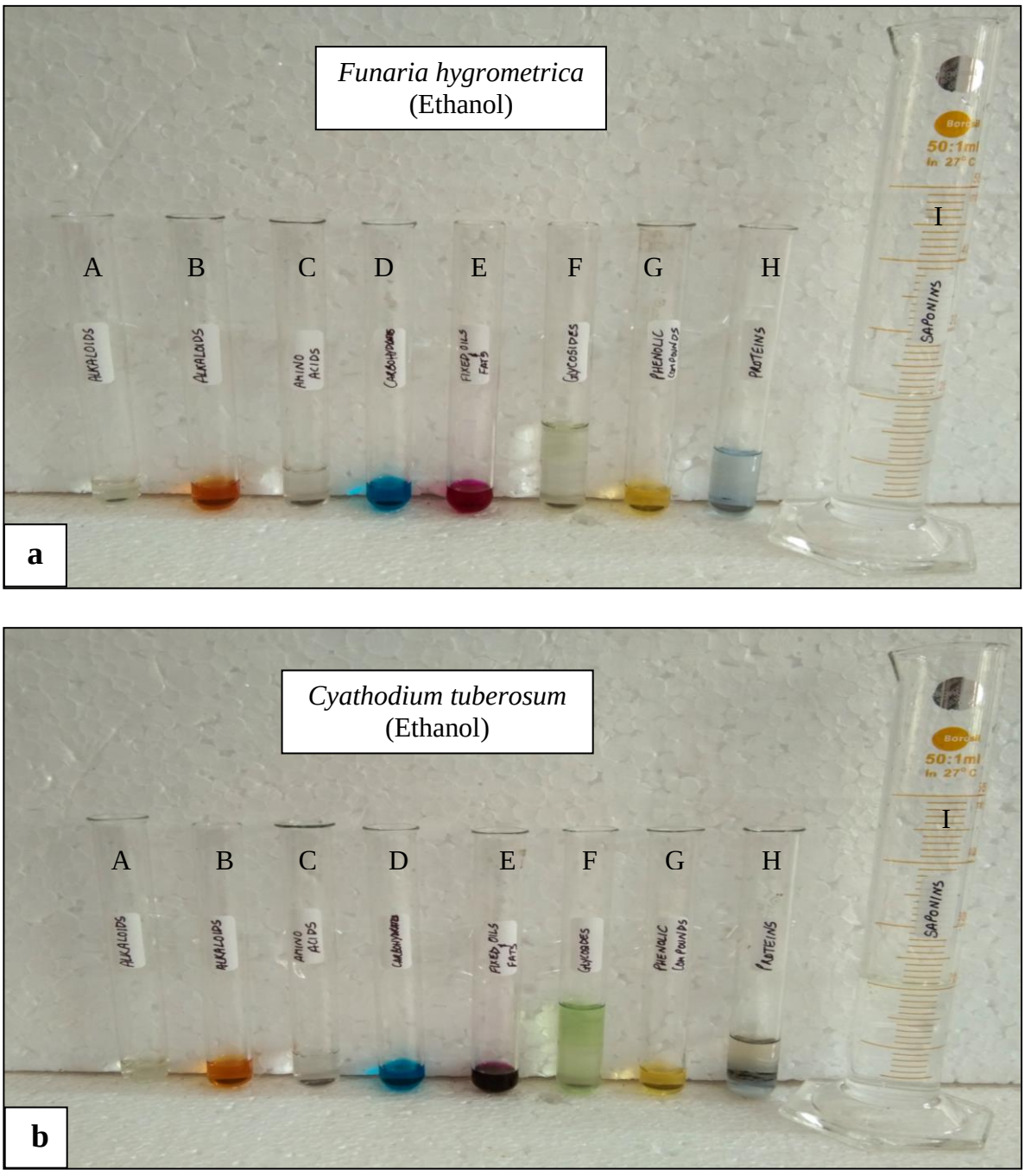


Figure 4: Preliminary phytochemical analysis of ethanolic extracts of Bryophytes (a) *Funaria hygrometrica* and (b) *Cyathodium tuberosum*. A. Mayer's test for detection of Alkaloids; B. Wagner's test for detection of Alkaloids; C. Ninhydrin test for detection of Amino acids; D. Benedict's test for detection of Carbohydrates; E. Saponification test for detection of Fixed oils and Fats; F. Borntrager's test for detection of Glycosides; G. Ferric chloride test for detection of Phenolic compounds; H. Biuret test for detection of Proteins; I. Detection of Saponins.

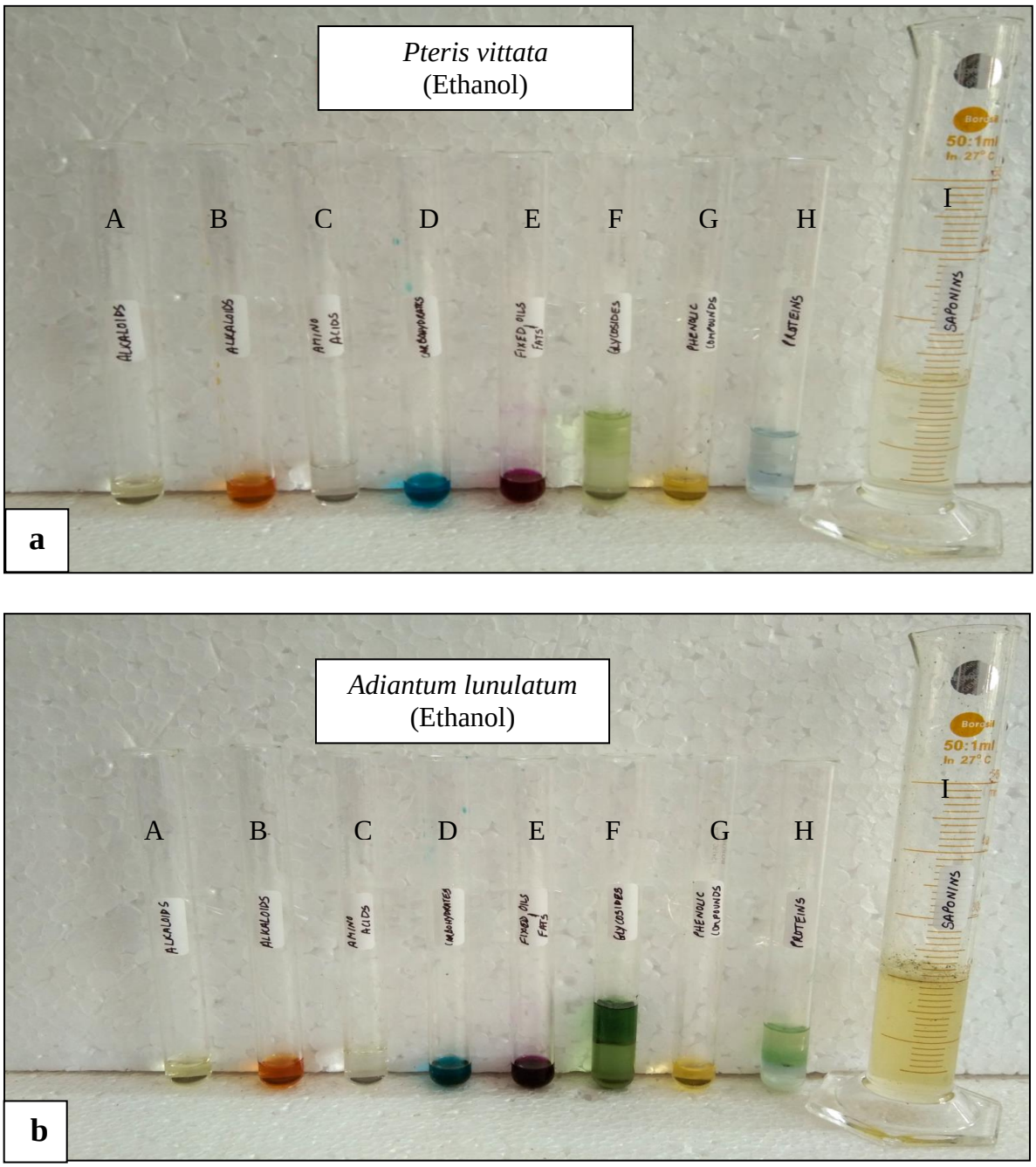


Figure 5: Preliminary phytochemical analysis of ethanolic extracts of Pteridophytes (a) *Pteris vittata* and (b) *Adiantum lunulatum*. A. Mayer's test for detection of Alkaloids; B. Wagner's test for detection of Alkaloids; C. Ninhydrin test for detection of Amino acids; D. Benedict's test for detection of Carbohydrates; E. Saponification test for detection of Fixed oils and Fats; F. Borntrager's test for detection of Glycosides; G. Ferric chloride test for detection of Phenolic compounds; H. Biuret test for detection of Proteins; I. Detection of Saponins.

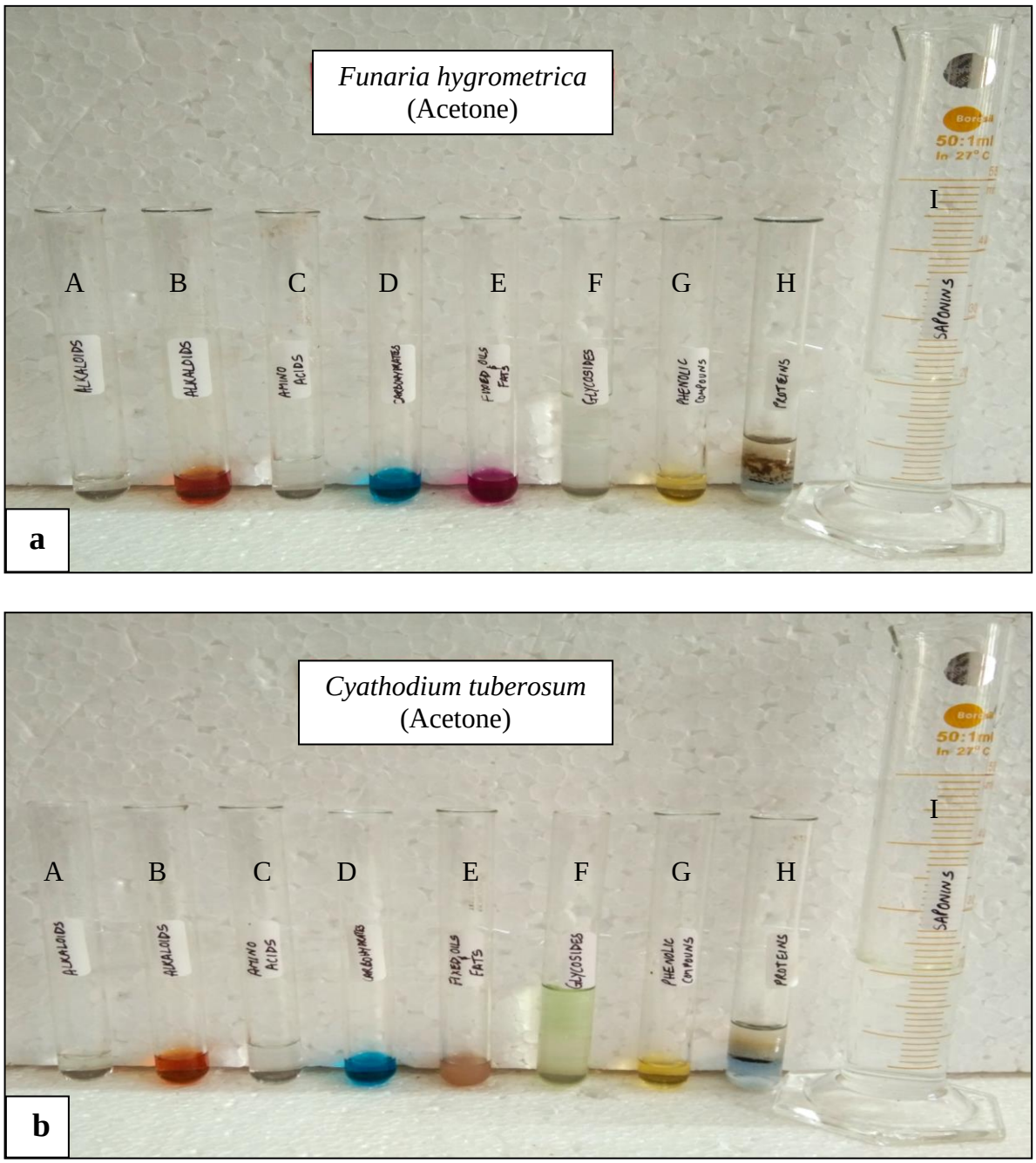


Figure 6: Preliminary phytochemical analysis of acetone extracts of Bryophytes (a) *Funaria hygrometrica* and (b) *Cyathodium tuberosum*. A. Mayer's test for detection of Alkaloids; B. Wagner's test for detection of Alkaloids; C. Ninhydrin test for detection of Amino acids; D. Benedict's test for detection of Carbohydrates; E. Saponification test for detection of Fixed oils and Fats; F. Borntrager's test for detection of Glycosides; G. Ferric chloride test for detection of Phenolic compounds; H. Biuret test for detection of Proteins; I. Detection of Saponins.

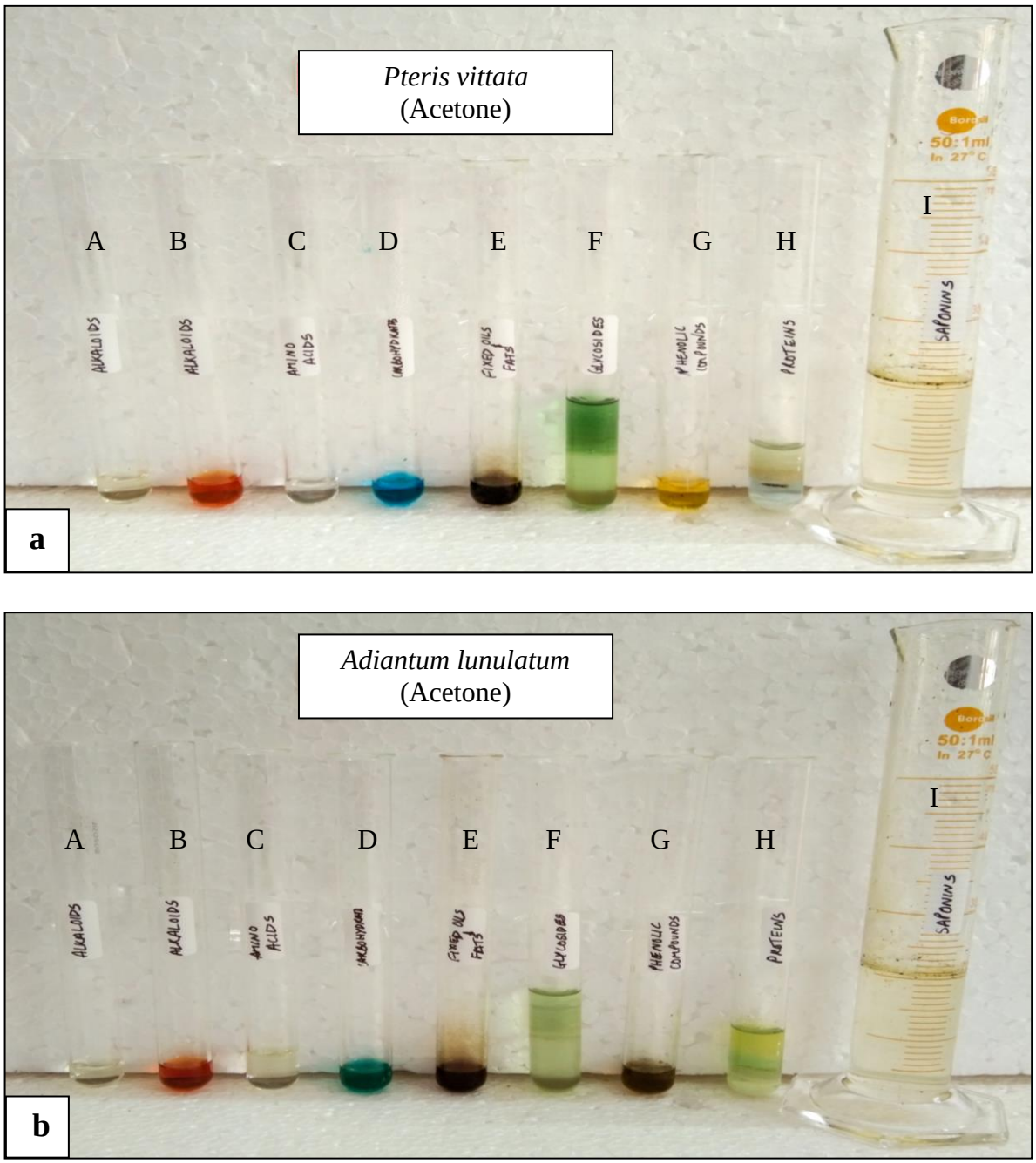
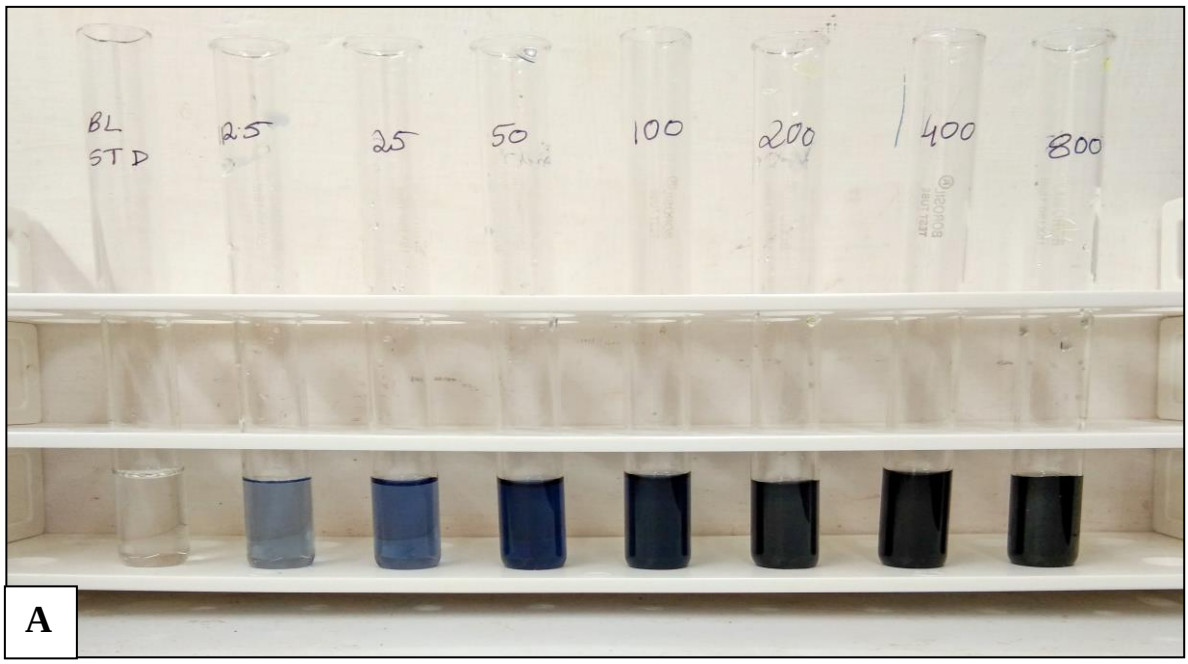


Figure 7: Preliminary phytochemical analysis of acetone extracts of Pteridophytes (a) *Pteris vittata* and (b) *Adiantum lunulatum*. A. Mayer's test for detection of Alkaloids; B. Wagner's test for detection of Alkaloids; C. Ninhydrin test for detection of Amino acids; D. Benedict's test for detection of Carbohydrates; E. Saponification test for detection of Fixed oils and Fats; F. Borntrager's test for detection of Glycosides; G. Ferric chloride test for detection of Phenolic compounds; H. Biuret test for detection of Proteins; I. Detection of Saponins.

Table No. 4: The amount of Total Phenolic Content (TPC) in methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Plant extracts		TPC (mg of GAE/g of extract)
Methanol	*Funaria hygrometrica*	3.697 ± 0.007
	Cyathodium tuberosum	8.495 ± 0.007
	Pteris vittata	**13.43 ± 0.02**
	Adiantum lunulatum	10.48 ± 0.01
Ethanol	*Funaria hygrometrica*	1.829 ± 0.001
	Cyathodium tuberosum	6.318 ± 0.01
	Pteris vittata	**9.420 ± 0.02**
	Adiantum lunulatum	7.724 ± 0.003
Acetone	*Funaria hygrometrica*	3.678 ± 0.01
	Cyathodium tuberosum	7.879 ± 0.02
	Pteris vittata	11.67 ± 0.02
	Adiantum lunulatum	**16.20 ± 0.01**

GAE: Gallic Acid Equivalent; Results are reported as mean ± standard deviation of triplicate measurements.



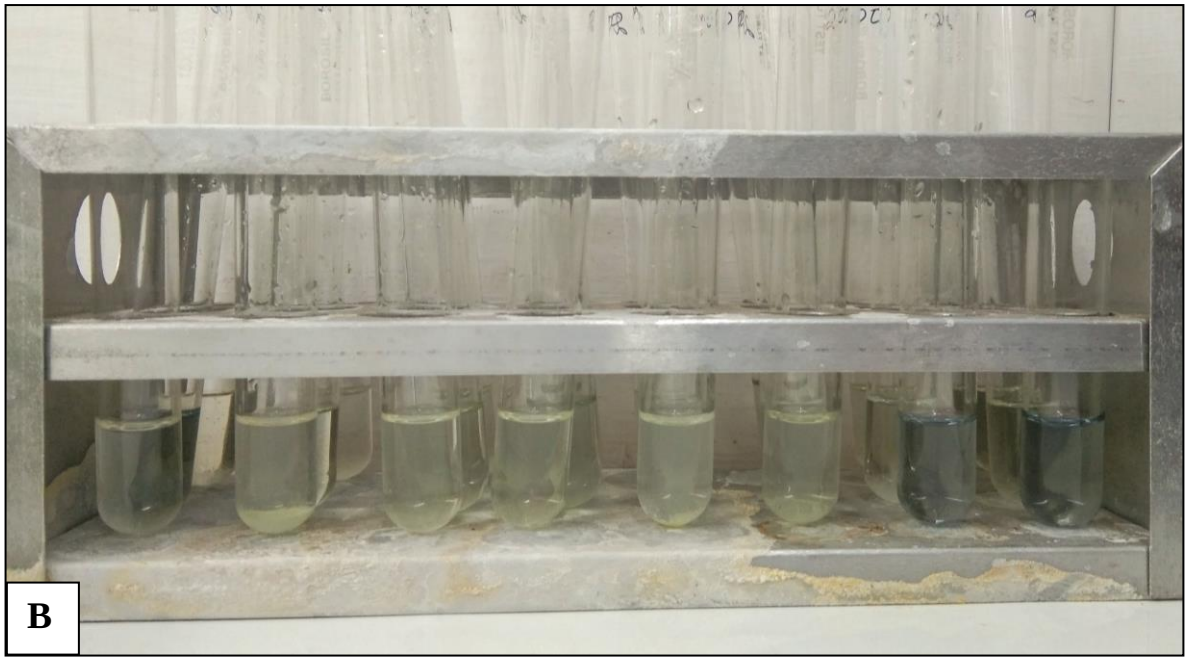


Figure 8: Determination of Total Phenolic Content (TPC) of (A.) Standard Gallic acid and (B.) methanolic, ethanolic and acetone extracts of *Funaria hygrometrica*, *Cyathodium tuberosum*, *Pteris vittata* and *Adiantum lunulatum*. The percent (%) of Total Phenol Content as visible by the colour difference in test-tubes during assay.

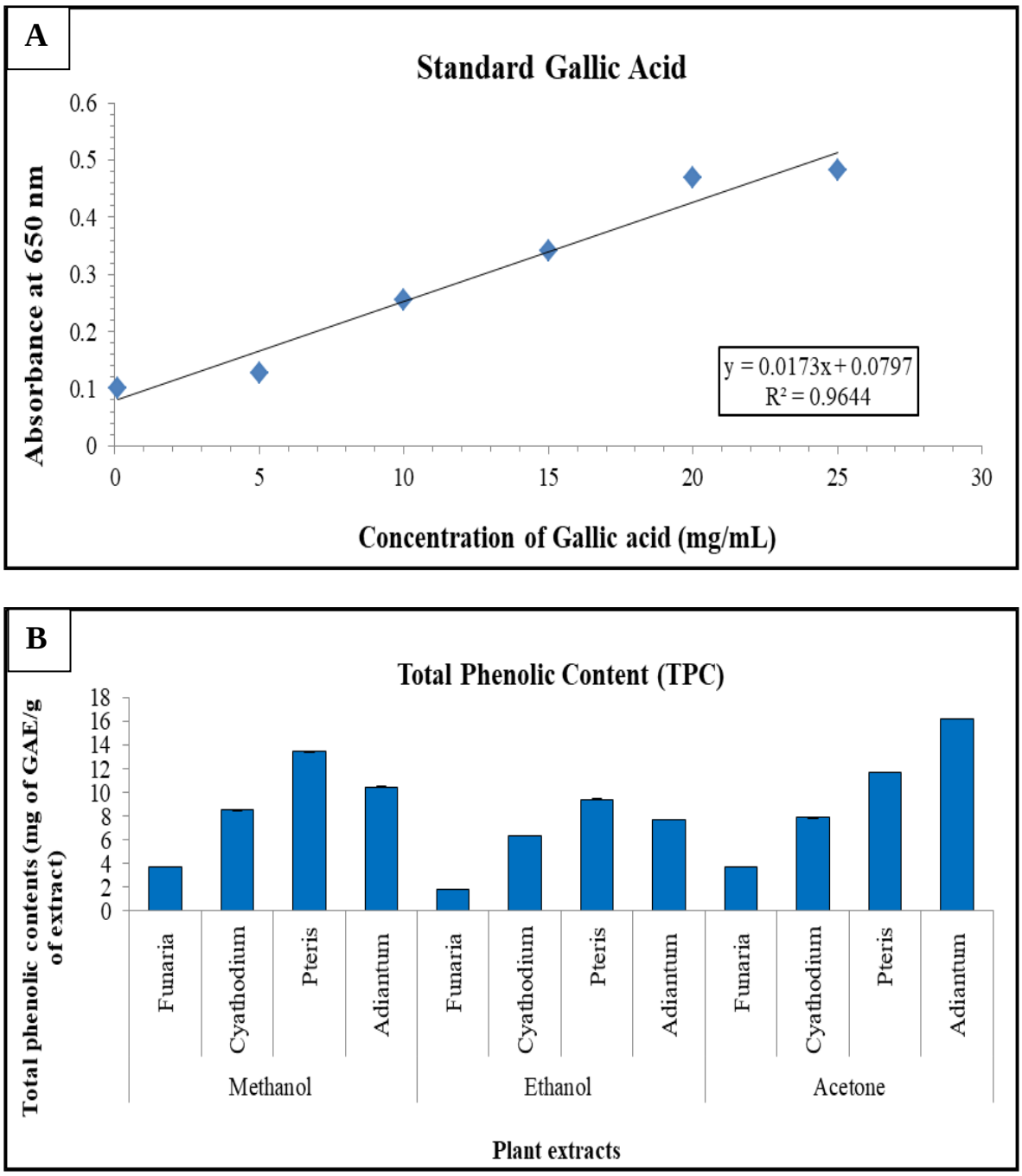


Figure 9: (A) Calibration curve for Gallic Acid (mg/mL); (B) Total phenolic content (mg of Gallic acid equivalent (GAE) /g of extract).

Table No. 5: DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and methanolic extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr. No.	Concentration (µg/mL)	L - Ascorbic acid	Methanolic extracts			
			Funaria hygrometrica	*Cyathodium tuberosum*	*Pteris vittata*	*Adiantum lunulatum*
1.	12.5	13.30 ± 0.01	0.434 ± 0.002	0.592 ± 0.003	4.623 ± 0.01	3.605 ± 0.007
2.	25	26.99 ± 0.02	0.592 ± 0.002	0.790 ± 0.005	7.549 ± 0.01	6.107 ± 0.009
3.	50	45.66 ± 0.03	1.303 ± 0.007	1.066 ± 0.002	25.74 ± 0.08	14.08 ± 0.008
4.	100	64.49 ± 0.07	1.343 ± 0.01	2.133 ± 0.002	46.90 ± 0.02	28.84 ± 0.004
5.	200	97.08 ± 0.005	3.476 ± 0.003	5.055 ± 0.006	89.19 ± 0.004	57.21 ± 0.02

Results are reported as % Inhibition ± standard deviation of triplicate measurements.

Table No. 6: DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and ethanolic extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr.No.	Concentration (µg/mL)	L - Ascorbic acid	Ethanolic extracts			
			Funaria hygrometrica	*Cyathodium tuberosum*	*Pteris vittata*	*Adiantum lunulatum*
1.	12.5	13.30 ± 0.01	0.809 ± 0.005	0.732 ± 0.004	0.041 ± 0.003	0.448 ± 0.001
2.	25	26.99 ± 0.02	1.416 ± 0.002	1.301 ± 0.002	0.737 ± 0.003	0.774 ± 0.004
3.	50	45.66 ± 0.03	1.537 ± 0.004	1.626 ± 0.010	1.147 ± 0.002	1.100 ± 0.002
4.	100	64.49 ± 0.07	1.780 ± 0.002	1.707 ± 0.004	3.686 ± 0.006	1.915 ± 0.002
5.	200	97.08 ± 0.005	2.549 ± 0.006	3.049 ± 0.003	8.149 ± 0.006	3.545 ± 0.004

Results are reported as % Inhibition ± standard deviation of triplicate measurements.

Table No. 7: DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr.No.	Concentration (µg/mL)	L - Ascorbic acid	Acetone extracts			
			Funaria hygrometrica	*Cyathodium tuberosum*	*Pteris vittata*	*Adiantum lunulatum*
1.	12.5	13.30 ± 0.01	0.753 ± 0.005	0.904 ± 0.001	0.851 ± 0.003	0.818 ± 0.005
2.	25	26.99 ± 0.02	0.942 ± 0.005	1.017 ± 0.004	0.889 ± 0.007	1.207 ± 0.001
3.	50	45.66 ± 0.03	1.318 ± 0.003	1.620 ± 0.003	1.121 ± 0.005	5.569 ± 0.02
4.	100	64.49 ± 0.07	1.695 ± 0.007	2.109 ± 0.009	2.204 ± 0.002	14.29 ± 0.05
5.	200	97.08 ± 0.005	2.561 ± 0.002	3.390 ± 0.01	6.071 ± 0.003	23.09 ± 0.02

Results are reported as % Inhibition ± standard deviation of triplicate measurements.

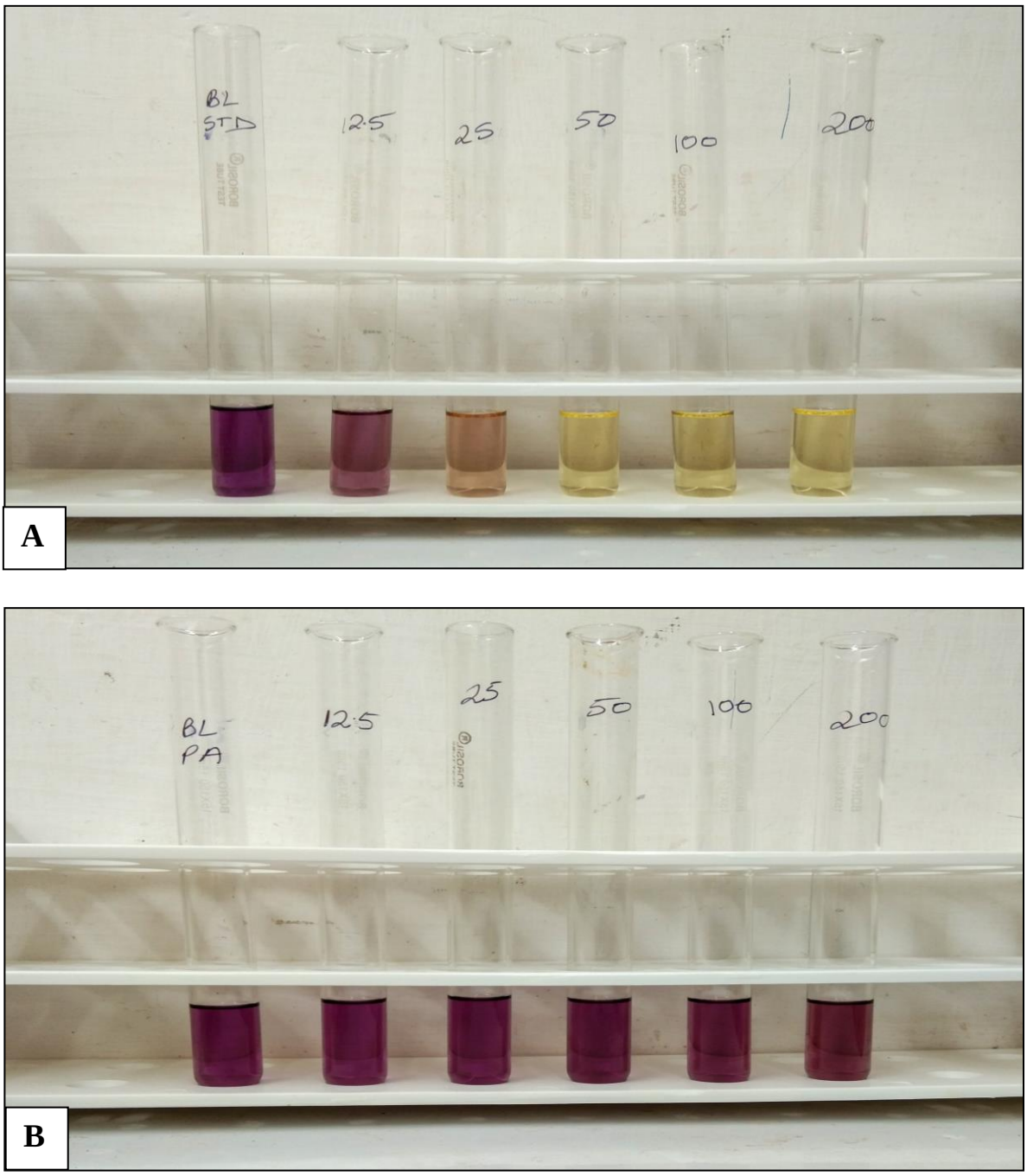


Figure 10: DPPH Radical scavenging activity assay of (A) Standard (L - Ascorbic acid) and (B) different dilutions of plant extract.

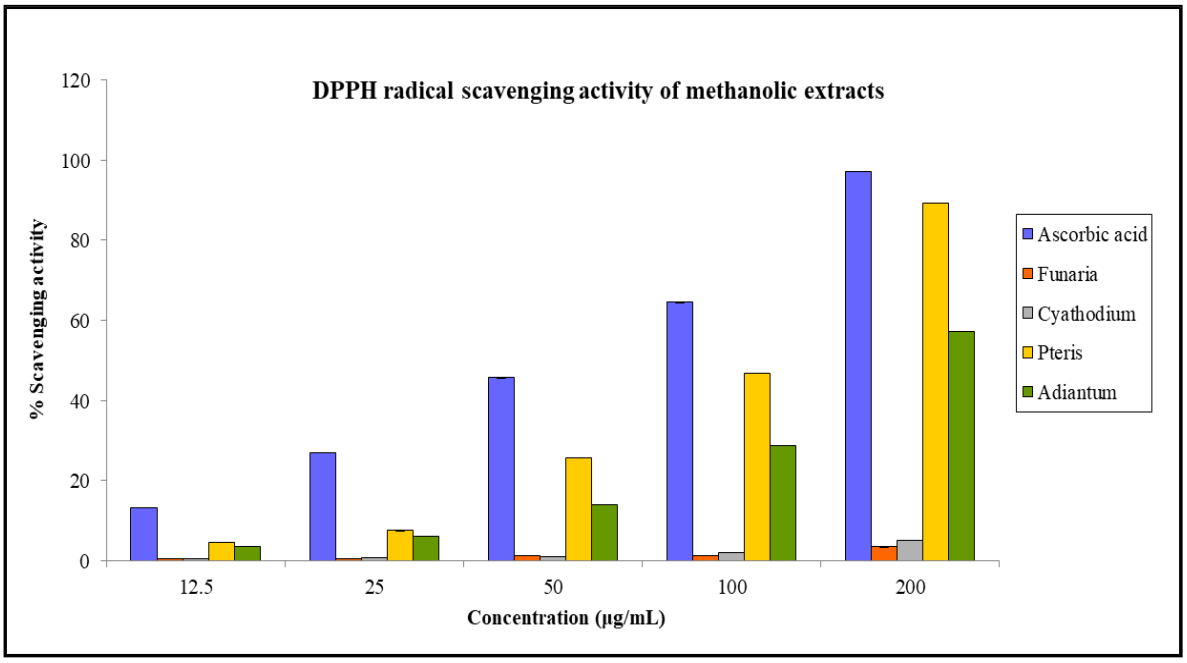


Figure 11: DPPH radical scavenging activity; Bar graph showing comparison of % DPPH radical scavenging activity between different concentrations of methanolic extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*), Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) and Standard (L - Ascorbic acid).

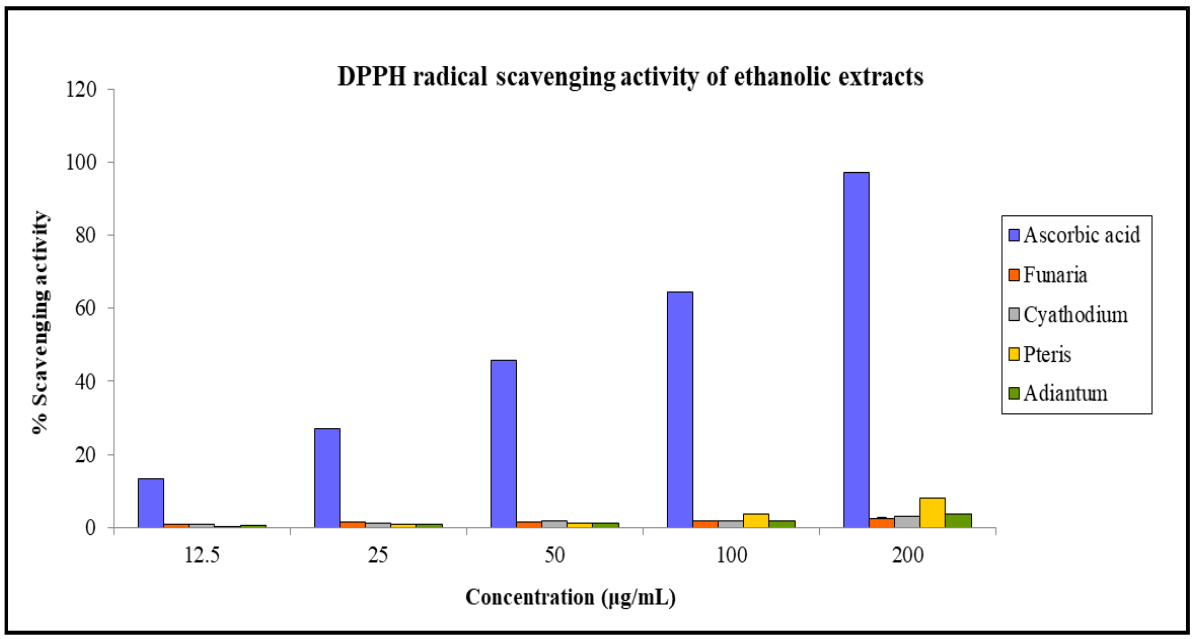


Figure 12: DPPH radical scavenging activity; Bar graph showing comparison of % DPPH radical scavenging activity between different concentrations of ethanolic extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*), Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) and Standard (L - Ascorbic acid).

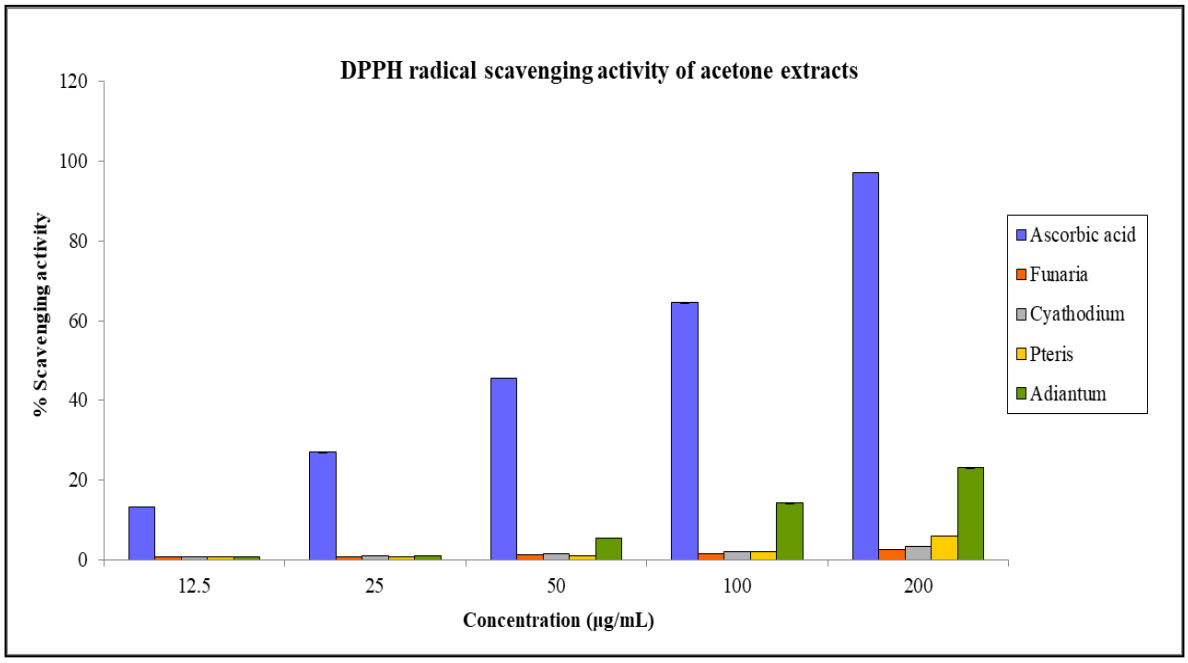


Figure 13: DPPH radical scavenging activity; Bar graph showing comparison of % DPPH radical scavenging activity between different concentrations of acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*), Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) and Standard (L - Ascorbic acid).

Table No. 8: IC_{50} values of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) with standard (L - Ascorbic acid).

Sr. No.	Samples / Standard	IC_{50} values (µg/mL)		
		Methanolic samples	**Ethanolic samples**	**Acetone samples**
1.	*Funaria hygrometrica*	3211.15	6201.52	5297.87
2.	*Cyathodium tuberosum*	2080.52	4551.37	3728.20
3.	*Pteris vittata*	**110.91**	**1166.11**	1759.65
4.	*Adiantum lunulatum*	174.57	3067.98	**408.45**
5.	L - Ascorbic acid (Standard)	78.67		

Table No. 9: Antifungal activity of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) on fungal (*Aspergillus* sp.) strain by disc diffusion method.

Plant extracts/ positive and negative control	**Antifungal activity (*Aspergillus* sp.)**		
	Methanol	**Ethanol**	**Acetone**
Funaria hygrometrica	+	+	+
Cyathodium tuberosum	+	+	+
Pteris vittata	++	++	++
Adiantum lunulatum	++	++	++
Ketoconazole (Positive control)	++	++	++
100% Methanol/ Ethanol/ Acetone (Negative control)	-	-	-

No inhibition (-), Less inhibition (+), More inhibition (++)

Table No. 10: Antibacterial activity of methanolic, ethanolic and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*) on bacterial (*Escherichia coli*) strain by disc diffusion method.

Plant extracts/ positive and negative control	**Antibacterial activity (*Escherichia coli*)**		
	Methanol	**Ethanol**	**Acetone**
Funaria hygrometrica	-	-	-
Cyathodium tuberosum	-	-	-
Pteris vittata	+	-	+
Adiantum lunulatum	+	+	-
Gentamicin (Positive control)	++	++	++
100% Methanol/ Ethanol/ Acetone (Negative control)	-	-	-

No inhibition (-), Less inhibition (+), More inhibition (++)

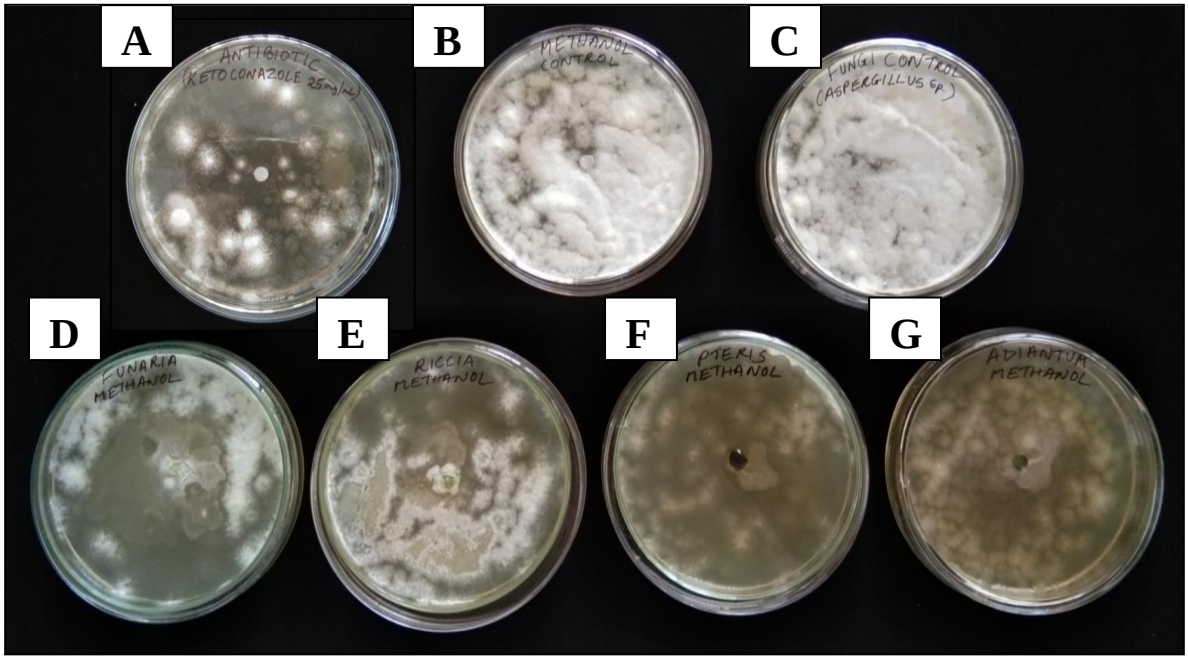


Figure 14: Antifungal activity of methanolic extracts of (D) *Funaria hygrometrica*, (E) *Cyathodium tuberosum*, (F) *Pteris vittata*, (G) *Adiantum lunulatum* and (A) positive and (B) negative control against (C) fungal (*Aspergillus* sp.) strain.

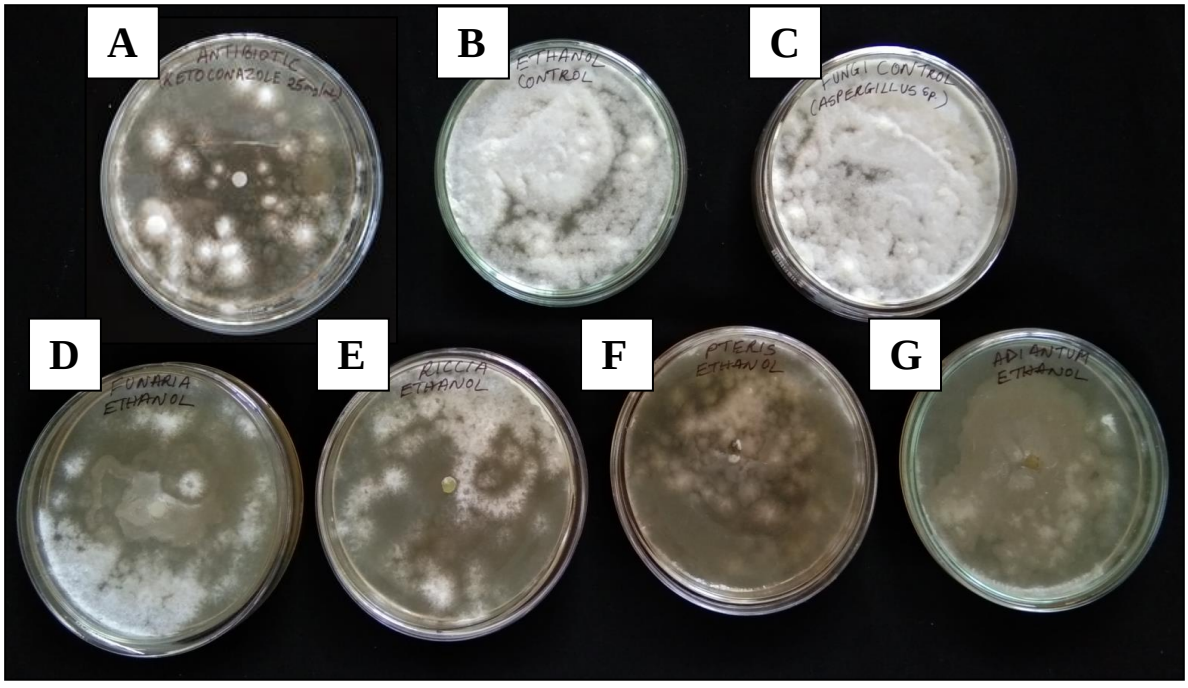


Figure 15: Antifungal activity of ethanolic extracts of (D) *Funaria hygrometrica*, (E) *Cyathodium tuberosum*, (F) *Pteris vittata*, (G) *Adiantum lunulatum* and (A) positive and (B) negative control against (C) fungal (*Aspergillus* sp.) strain.

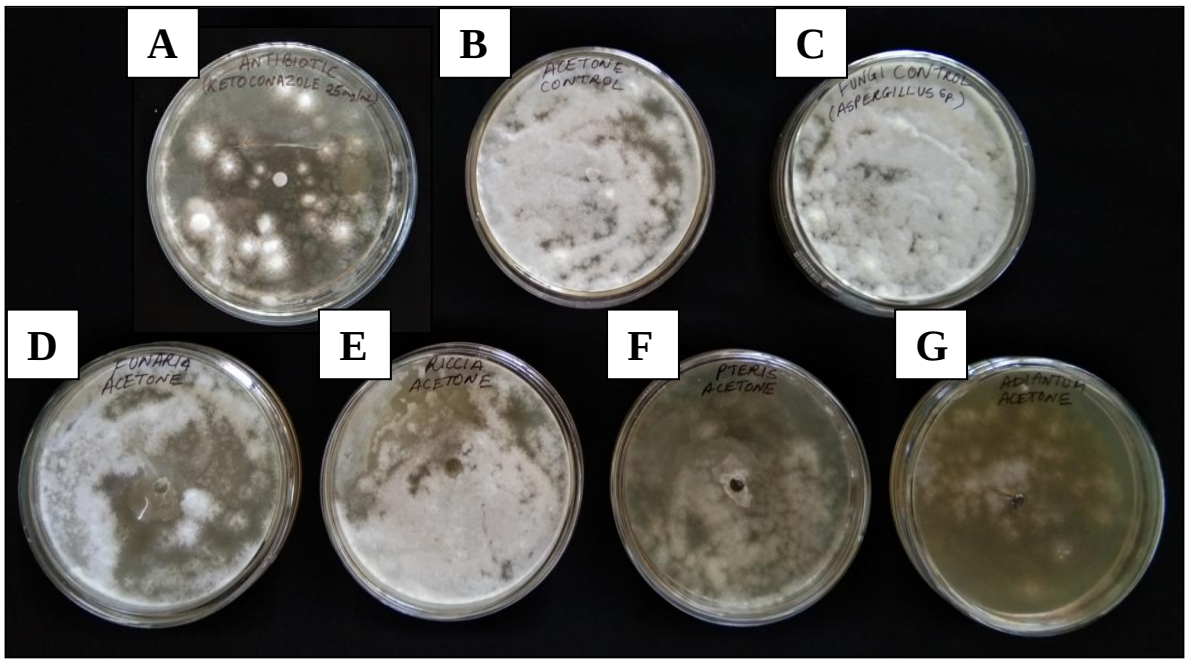


Figure 16: Antifungal activity of acetone extracts of (D) *Funaria hygrometrica*, (E) *Cyathodium tuberosum*, (F) *Pteris vittata*, (G) *Adiantum lunulatum* and (A) positive and (B) negative control against (C) fungal (*Aspergillus* sp.) strain.

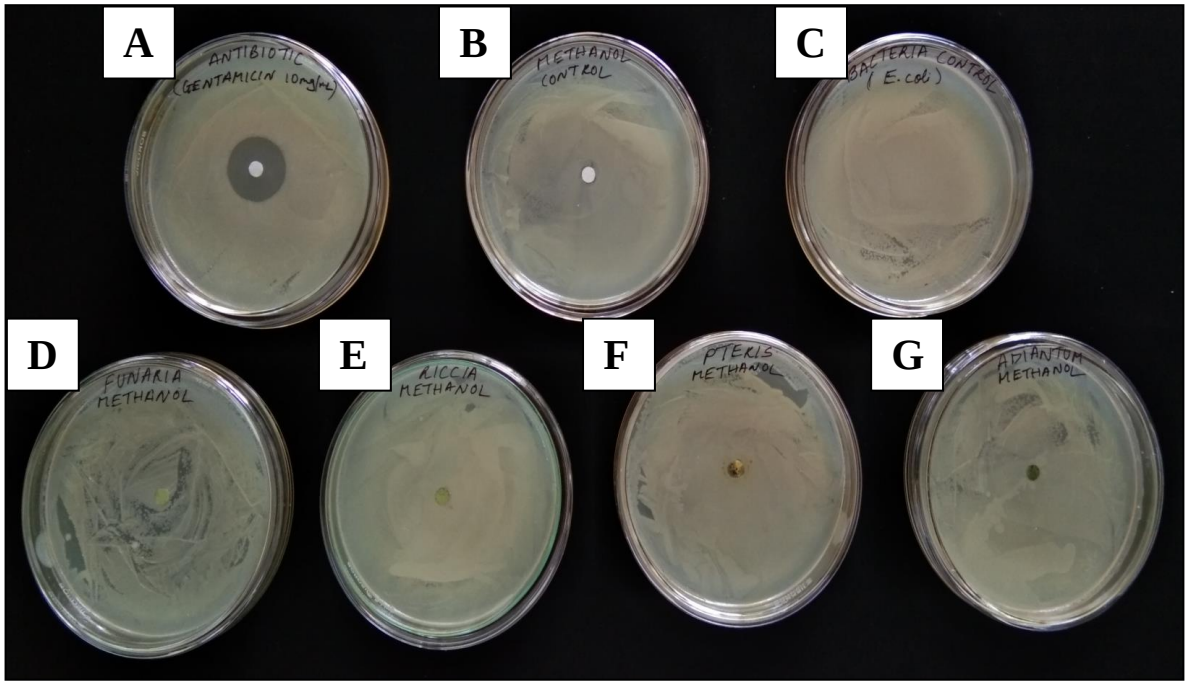


Figure 17: Antibacterial activity of methanolic extracts of (D) *Funaria hygrometrica*, (E) *Cyathodium tuberosum*, (F) *Pteris vittata*, (G) *Adiantum lunulatum* and (A) positive and (B) negative control against (C) bacterial (*Escherichia coli*) strain.

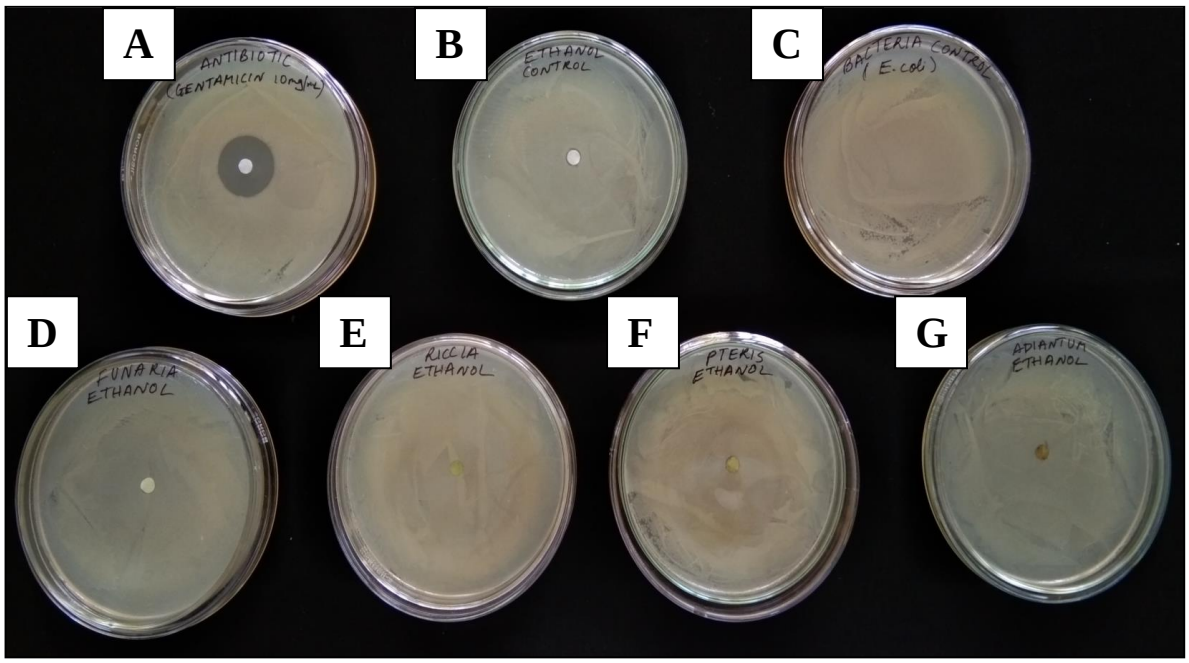


Figure 18: Antibacterial activity of ethanolic extracts of (D) *Funaria hygrometrica*, (E) *Cyathodium tuberosum*, (F) *Pteris vittata*, (G) *Adiantum lunulatum* and (A) positive and (B) negative control against (C) bacterial (*Escherichia coli*) strain.

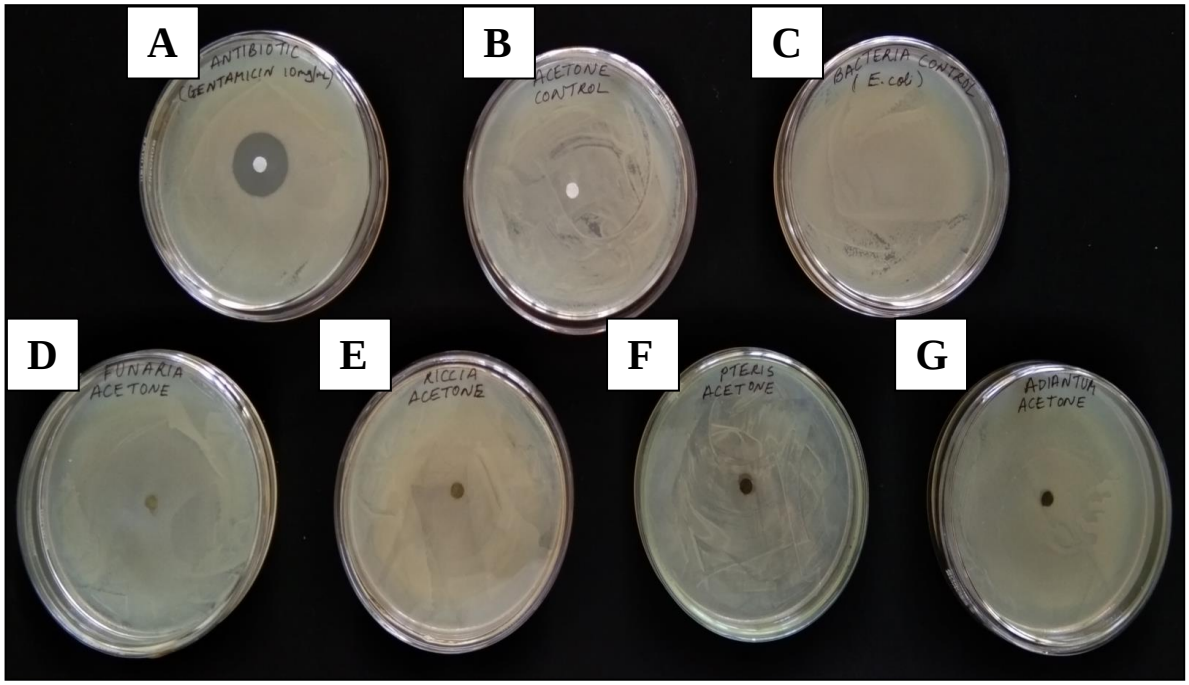


Figure 19: Antibacterial activity of acetone extracts of (D) *Funaria hygrometrica*, (E) *Cyathodium tuberosum*, (F) *Pteris vittata*, (G) *Adiantum lunulatum* and (A) positive and (B) negative control against (C) bacterial (*Escherichia coli*) strain.

Table No. 11: Rf values and spectral properties of phenolic compounds showing respective colouration under visible light and long UV in methanolic extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr.No.	Methanolic Plant extracts	Rf × 100	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max}	Phenolic compounds (Harborne, 1973)
1.	*Funaria hygrometrica*	-	-	-	265	p-Hydroxybenzoic
2.	*Cyathodium tuberosum*	0.88 -	- -	Blue -	- 265	Salicylic p-Hydroxybenzoic
3.	*Pteris vittata*	0.12 0.23 0.43 0.68 0.86 0.93	Blue Brick red Bluish-pink - - -	Blue Brick red Bluish-pink Blue Blue Blue	- - - - - -	Protocatechuic 4-Methylresorcinol 2- Methylresorcinol Syringic Vanillic Salicylic
4.	*Adiantum lunulatum*	0.15 0.23 0.49 0.79 0.87 0.93	Blue Brick red - - - -	Blue Brick red Blue Blue Blue Blue	260 - - - - -	Protocatechuic 4-Methylresorcinol p-Hydroxybenzoic Syringic Vanillic Salicylic

Table No. 12: Rf values and spectral properties of phenolic compounds showing respective colouration under visible light and long UV in ethanolic extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr.No.	Ethanolic Plant extracts	Rf × 100	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max}	Phenolic compounds (Harborne, 1973)
1.	*Funaria hygrometrica*	-	-	-	265	p-Hydroxybenzoic
2.	*Cyathodium tuberosum*	0.77 0.84 -	- - -	Blue Blue -	- - 265	Syringic Vanillic p-Hydroxybenzoic
3.	*Pteris vittata*	0.14 0.21 0.33 0.59 0.73 0.85 -	Red Brick red - - - - -	Red Brick red Blue Blue Blue Blue -	- - - - - - 266	Resorcinol 4-Methylresorcinol Gentisic p-Hydroxybenzoic Syringic Vanillic Pyrogallol
4.	*Adiantum lunulatum*	0.14 0.21 0.34 0.42 0.76 0.87	Red Brick red - Bluish-pink - -	Red Brick red Blue Bluish-pink Blue Blue	- - - - - -	Resorcinol 4-Methylresorcinol Gentisic 2- Methylresorcinol Syringic Vanillic

Table No. 13: Rf values and spectral properties of phenolic compounds showing respective colouration under visible light and long UV in acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr.No.	Acetone Plant extracts	Rf × 100	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max}	Phenolic compounds (Harborne, 1973)
1.	*Funaria hygrometrica*	0.92	-	Blue	-	Salicylic
2.	*Cyathodium tuberosum*	0.93	-	Blue	-	Salicylic
3.	*Pteris vittata*	0.10 0.34 0.44 0.52 0.78 0.87 0.95	- - Brick red - - - -	- - Brick red Blue Blue Blue Blue	- - - - - - -	Pyrogallol Catechol 2- Methylresorcinol p-Hydroxybenzoic Syringic Vanillic Salicylic
4.	*Adiantum lunulatum*	0.09 0.25 0.36 0.45 0.52 0.72 0.87 0.94	- Brick red - Bluish-pink - - - -	- Brick red - Bluish-pink Blue Blue Blue Blue	- - - - - - - -	Pyrogallol 4-Methylresorcinol Catechol 2- Methylresorcinol p-Hydroxybenzoic Syringic Vanillic Salicylic

Table No. 14: Rf values and spectral properties of flavonoid compounds showing respective colouration under visible light and long UV in methanolic extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr.No.	Methanolic Plant extracts	Rf × 100	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max}	Flavonoid compounds (Harborne, 1973)
1.	*Funaria hygrometrica*	0.79 0.84 0.93	Dull ochre Bright yellow Dull brown	Dull ochre Bright yellow Dull brown	- - -	Luteolin Kaempferol Kayaflavone
2.	*Cyathodium tuberosum*	0.84 0.93	Bright yellow Dull brown	Bright yellow Dull brown	- -	Kaempferol Kayaflavone
3.	*Pteris vittata*	0.33 0.47 0.65 0.74 0.86 0.94	Dull ochre Fluorescent yellow Bright yellow Bright yellow Dull ochre Dull brown	Dull ochre Fluorescent yellow Bright yellow Bright yellow Dull ochre Dull brown	- - - - - -	Orientin Azaleatin Quercetin Isorhamnetin Apigenin Kayaflavone
4.	*Adiantum lunulatum*	0.48 0.57 0.66 0.79 0.84 0.95 -	Fluorescent yellow Dull ochre Bright yellow Dull ochre Bright yellow Dull brown -	Fluorescent yellow Dull ochre Bright yellow Dull ochre Bright yellow Dull brown -	- - - - - - 262	Azaleatin Isovitexin Quercetin Luteolin Kaempferol Kayaflavone Gossypetin

Table No. 15: Rf values and spectral properties of flavonoid compounds showing respective colouration under visible light and long UV in ethanolic extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata*, *Adiantum lunulatum*).

Sr.No.	Ethanolic Plant extracts	Rf × 100	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max}	Flavonoid compounds (Harborne, 1973)
1.	*Funaria hygrometrica*	0.78 0.84	Dull ochre Bright yellow	Dull ochre Bright yellow	- -	Luteolin Kaempferol
2.	*Cyathodium tuberosum*	0.77 0.85 0.89	Dull ochre Bright yellow Dull ochre	Dull ochre Bright yellow Dull ochre	- - -	Luteolin Kaempferol Apigenin
3.	*Pteris vittata*	0.29 0.75 0.78 0.85 0.89	Dull ochre Bright yellow Dull ochre Bright yellow Dull ochre	Dull ochre Bright yellow Dull ochre Bright yellow Dull ochre	- - - - -	Orientin Isorhamnetin Luteolin Kaempferol Apigenin
4.	*Adiantum lunulatum*	0.30 0.47 0.51 0.66 0.75 0.78 0.84	Dull ochre Fluorescent yellow Dull ochre Bright yellow Bright yellow Dull ochre Bright yellow	Dull ochre Fluorescent yellow Dull ochre Bright yellow Bright yellow Dull ochre Bright yellow	- - - - - - -	Orientin Azaleatin Isovitexin Quercetin Isorhamnetin Luteolin Kaempferol

Table No. 16: Rf values and spectral properties of flavonoid compounds showing respective colouration under visible light and long UV in acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr.No.	Acetone Plant extracts	Rf × 100	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max}	Flavonoid compounds (Harborne, 1973)
1.	*Funaria hygrometrica*	0.81 0.90 0.97	Dull ochre Dull ochre Dull brown	Dull ochre Dull ochre Dull brown	- - -	Chrysoeriol Apigenin Kayaflavone
2.	*Cyathodium tuberosum*	0.74 0.80 0.85 0.89 0.98	Bright yellow Dull ochre Bright yellow Dull ochre Dull brown	Bright yellow Dull ochre Bright yellow Dull ochre Dull brown	- - - - -	Isorhamnetin Chrysoeriol Kaempferol Apigenin Kayaflavone
3.	*Pteris vittata*	0.49 0.54 0.69 0.76 0.82 0.89 0.93	Fluorescent yellow Dull ochre Bright yellow Dull ochre Dull ochre Dull ochre Dull brown	Fluorescent yellow Dull ochre Bright yellow Dull ochre Dull ochre Dull ochre Dull brown	- - - - - - -	Azaleatin Isovitexin Quercetin Luteolin Chrysoeriol Apigenin Kayaflavone
4.	*Adiantum lunulatum*	0.64 0.77 0.86 0.96	Bright yellow Dull ochre Dull ochre Dull brown	Bright yellow Dull ochre Dull ochre Dull brown	- - - -	Quercetin Luteolin Apigenin Kayaflavone

Table No. 17: Rf values and spectral properties of photosynthetic pigments showing respective colouration in Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*).

Sr. No.	Rf × 100	Colour of spots	λ_{max}	Plant pigments (Maroti & Gabnai, 1971)			
				Funaria hygrometrica	*Cyathodium tuberosum*	*Pteris vittata*	*Adiantum lunulatum*
1.	0.68	Blue-green	430, 662	Chlorophyll a	Chlorophyll a	Chlorophyll a	Chlorophyll a
2.	0.54	Yellow-green	456, 645	Chlorophyll b	Chlorophyll b	Chlorophyll b	Chlorophyll b
3.	0.03	Light green	449, 635	-	-	Chlorophyll c	Chlorophyll c
4.	0.94	Yellow/Orange	452, 478	β-carotene	β-carotene	β-carotene	β-carotene
5.	0.85	Grey	409, 666	Phaeophytin a	Phaeophytin a	Phaeophytin a	Phaeophytin a
6.	0.51	Orange brown	446, 468	Fucoxanthin	Fucoxanthin	Fucoxanthin	-
7.	0.43	Yellow	428, 449, 479	-	-	-	Lutein
8.	0.22	Yellow	421, 442, 472	Violaxanthin	Violaxanthin	Violaxanthin	Violaxanthin
9.	0.08	Yellow	416, 440, 470	-	-	-	Neoxanthin

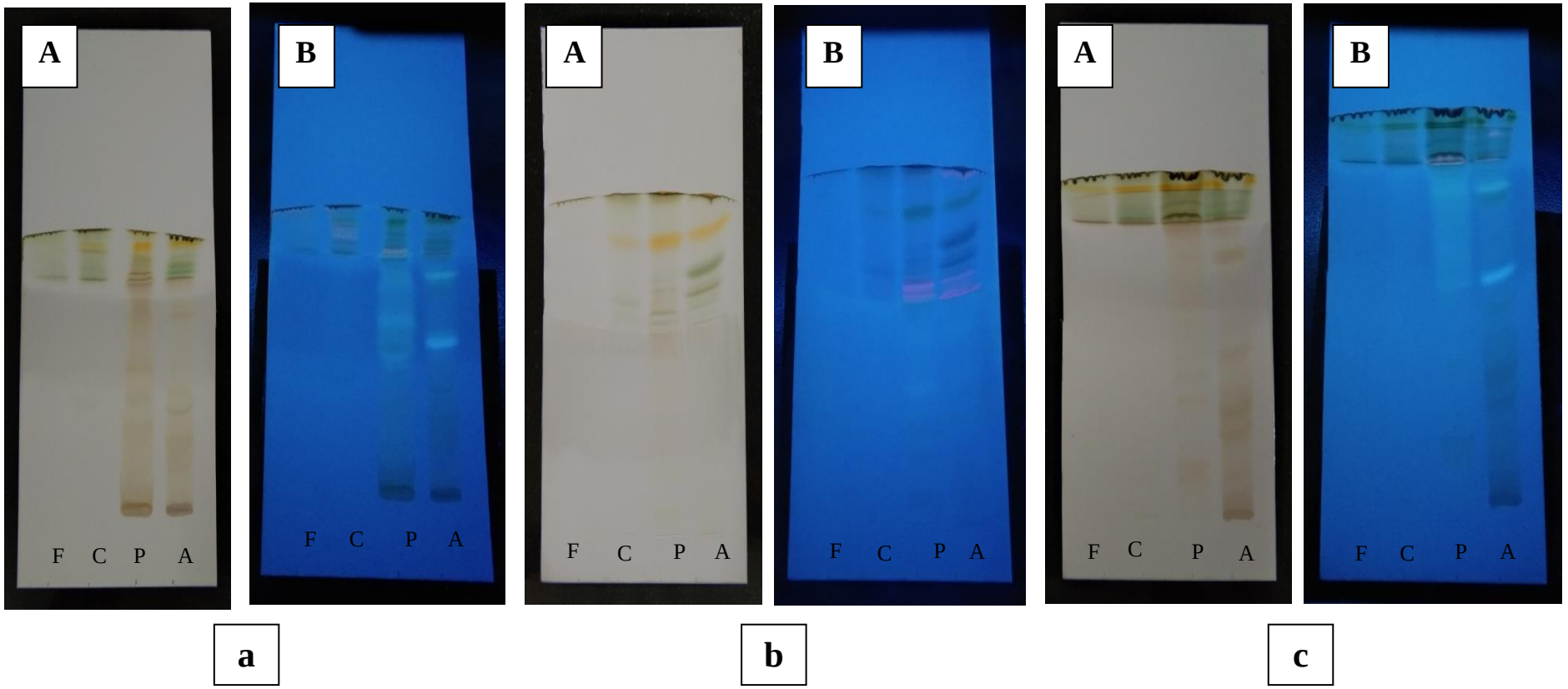


Figure 20: TLC analysis of phenolic compounds in methanol, ethanol and acetone extracts of Bryophytes (*Funaria hygrometrica, Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*). (A) Under visible light; (B) Under long UV at 365nm; (a) Methanol; (b) Ethanol; (c) Acetone; F- *Funaria hygrometrica*; C- *Cyathodium tuberosum*; P- *Pteris vittata*; A- *Adiantum lunulatum*.

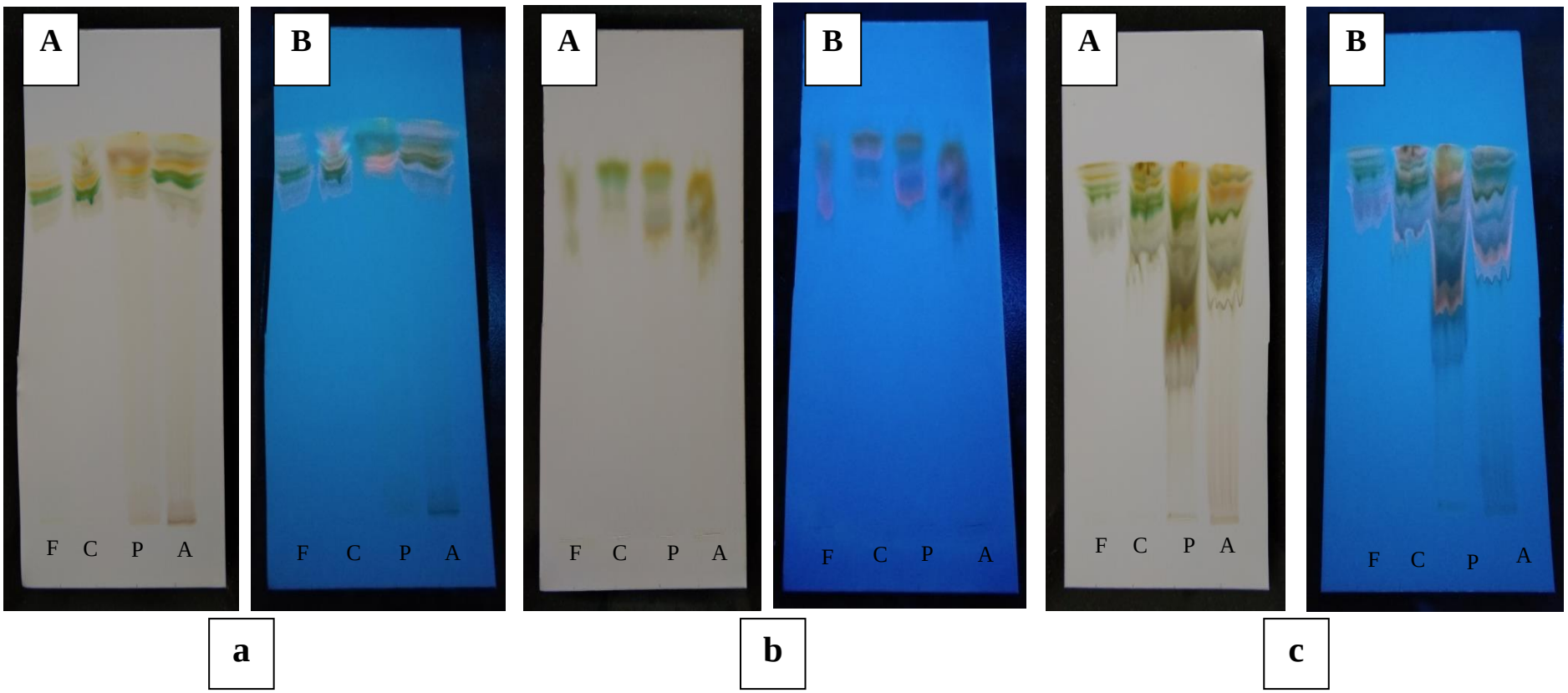


Figure 21: TLC analysis of flavonoid compounds in methanol, ethanol and acetone extracts of Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*). (A) Under visible light; (B) Under long UV at 365nm; (a) Methanol; (b) Ethanol; (c) Acetone; F- *Funaria hygrometrica*; C- *Cyathodium tuberosum*; P- *Pteris vittata*; A- *Adiantum lunulatum*.

Figure 22: TLC analysis of photosynthetic pigments in Bryophytes (*Funaria hygrometrica*, *Cyathodium tuberosum*) and Pteridophytes (*Pteris vittata, Adiantum lunulatum*). F- *Funaria hygrometrica*; C- *Cyathodium tuberosum*; P- *Pteris vittata*; A- *Adiantum lunulatum*.

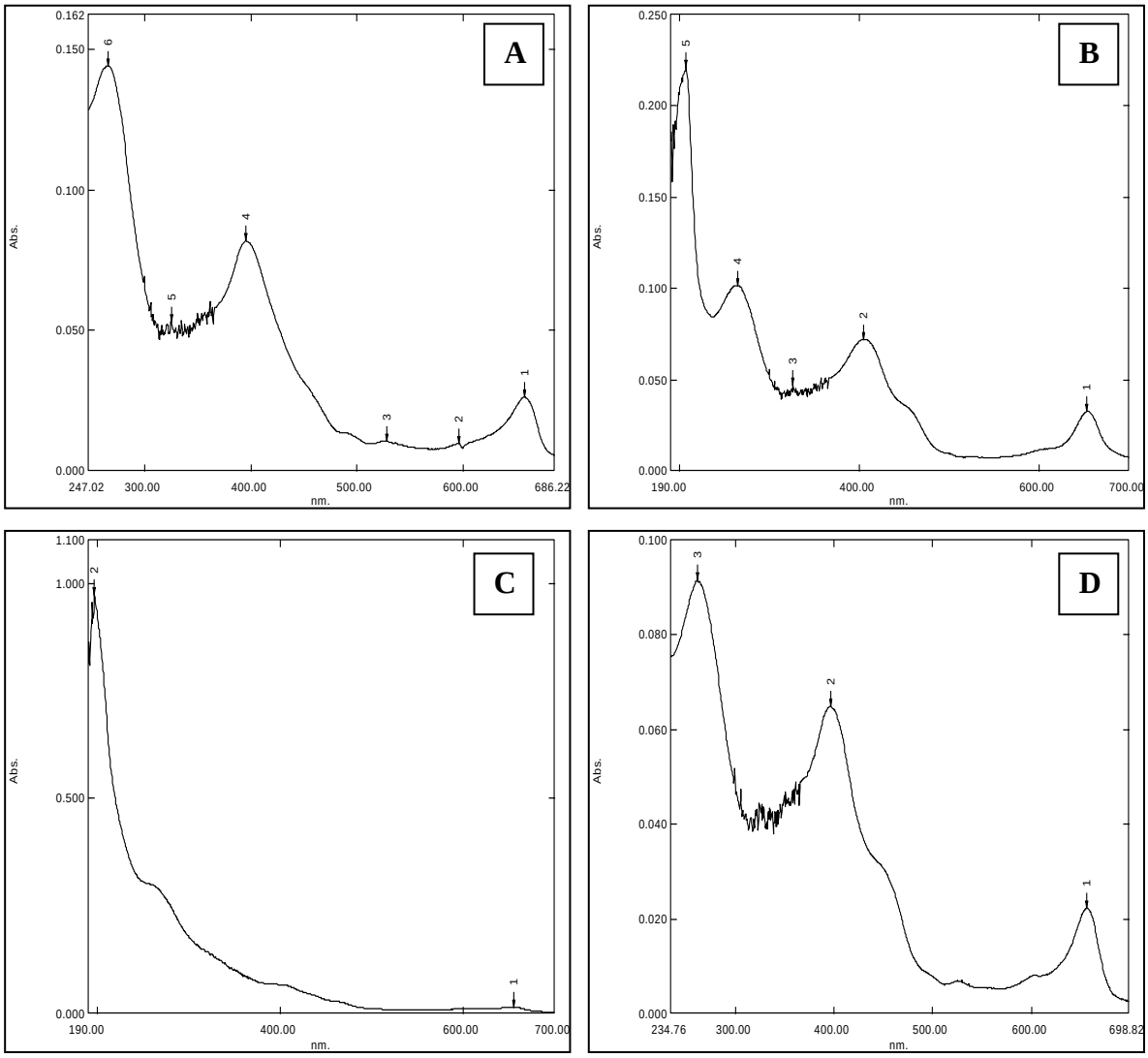


Figure 23: UV-VIS spectra (190-700 nm) of methanolic extracts of Bryophytes (A) *Funaria hygrometrica,* (B) *Cyathodium tuberosum* and Pteridophytes (C) *Pteris vittata,* (D) *Adiantum lunulatum*.

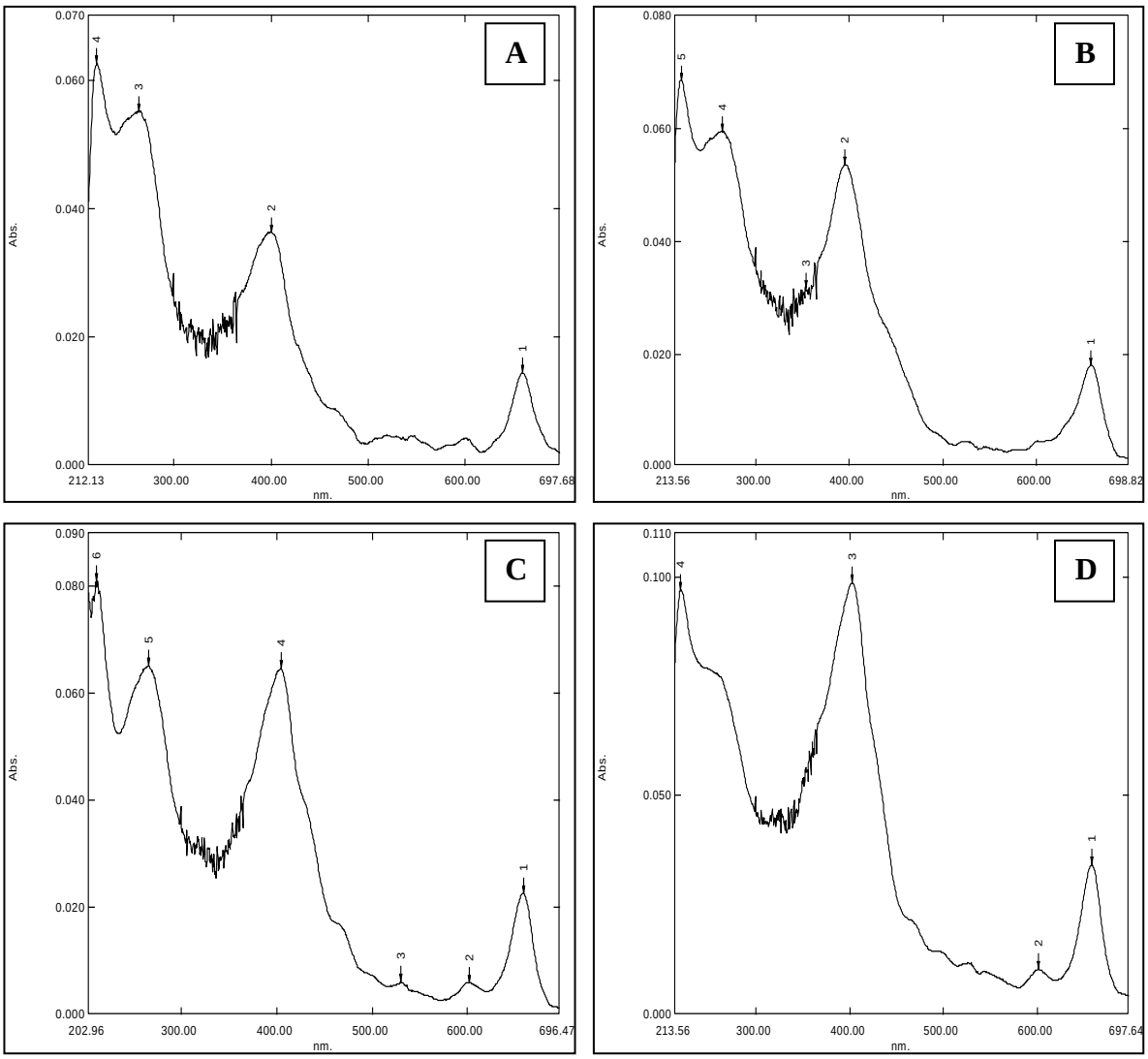


Figure 24: UV-VIS spectra (190-700 nm) of ethanolic extracts of Bryophytes (A) *Funaria hygrometrica,* (B) *Cyathodium tuberosum* and Pteridophytes (C) *Pteris vittata,* (D) *Adiantum lunulatum*.

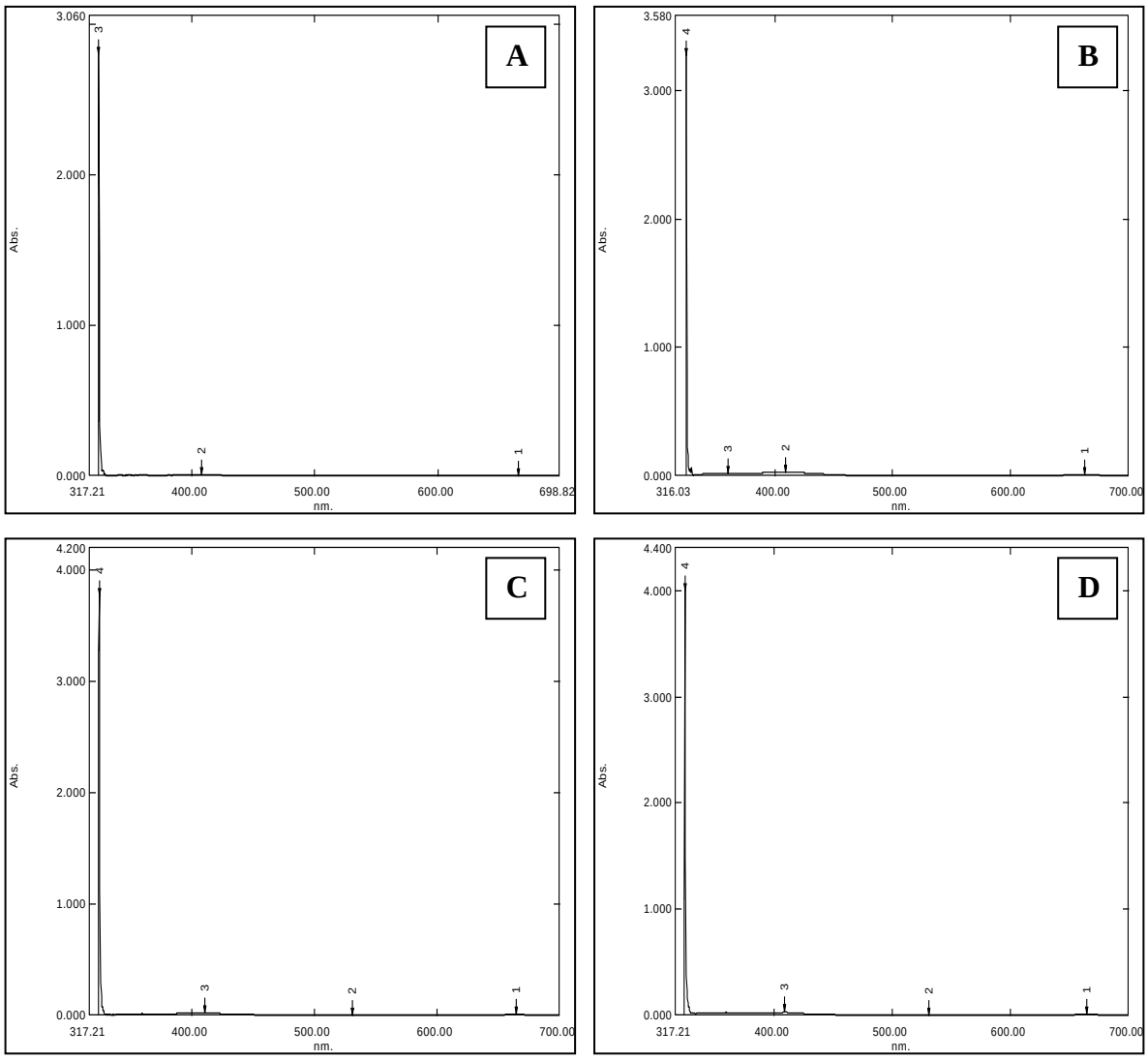


Figure 25: UV-VIS spectra (190-700 nm) of acetone extracts of Bryophytes (A) *Funaria hygrometrica,* (B) *Cyathodium tuberosum* and Pteridophytes (C) *Pteris vittata,* (D) *Adiantum lunulatum*.