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Chapter

Bryophytes: A Potential Source of Antioxidants

Dheeraj Gahtori and Preeti Chaturvedi

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Abstract

A variety of degenerative diseases are caused by free radicals. Oxidative stress, the major cause of the diseases, is due to the imbalance between the free radicals and the antioxidants. To overcome this imbalance, the body needs antioxidants whether endogenously present or supplied from exogenous sources. Hence, the search of effective natural antioxidants is greatly needed to fight the onset of degenerative diseases and aging. Indeed, vascular plants are well-known sources of good and efficient natural antioxidants. Non-tracheophytes are however relatively unexplored. Interestingly, these atracheophytes are endowed with the remarkable property of desiccation tolerance which makes them unique in the plant kingdom. The property is attributed to its specialized structure and rich reservoir of phytochemicals. Therefore, there is a need to bioprospect this rich resource for antioxidants.

Keywords: bryophytes, antimicrobial, antioxidant, phytochemicals

1. Introduction

The use of medicinal plants for treating human ailments is as old as the mankind. Man's keen observations of the mother nature led to disclosure of various curative properties of plants. These properties were acquired by the plants during evolution as adaptive strategies for protecting against various abiotic and biotic challenges faced by the plants. Changing climatic conditions of the earth also played an important role in designing plant's adaptive abilities. Human has utilized these abilities of the plants for ensuring his own survival. Needless to say, before the introduction of modern medicine, disease treatment was mainly managed by herbal remedies. Plants were found to be a rich source of therapeutic agents and hence contributed to the drug industry for a long time. Today also, many important medicinal compounds are derived from plant sources. There is huge potential to further harness this resource by exploring the vast diversity present in the plant world [1].

With increase in awareness and people becoming more health conscious, their attitude toward medicine and diet has undergone a dramatic transformation. Now, there is increased focus on plant-based diet and healthcare supplements. The natural supplements are relatively healthier and free from side effects of harmful chemicals. In human body, different natural mechanisms are responsible for production of free radicals and other reactive oxygen species (ROS). These species perform dual functions, viz., lethal as well as favorable, depending upon their concentrations. The delicate equilibrium between these two contrary effects needs to be maintained for a healthy life. At low or optimum levels, reactive oxygen species exert positive effects on cellular redox signaling and immune function, but at higher concentration, they produce oxidative stress, which may be responsible for onset of many degenerative diseases, apoptosis, aging, and food rancidity [2]. Therefore, wholesome antioxidant diet or natural antioxidant supplements should be used for a healthy life. Further, the novel and nonconventional sources of these antioxidants need to be documented regularly for relaxing the dependence on traditional sources on the one hand and for utilization of potential sources in the future as well.

Earlier, the food habits of man were ensuring sufficient intake of antioxidants in the form of fresh fruits, vegetables, and spices. In the fast-food age, change in food habits led to insufficient supply of these antioxidants. Now again, a need is being felt to use more and more of antioxidants in our day-to-day diet. As of today, both synthetic and natural antioxidants are very commonly used in food industry for increasing shelf life and improving quality of food. Another major industry using these antioxidants is medicine where they are mainly used for developing dietary supplements to promote health effect. Besides, cosmetic industry and herbal therapeutics are also using different types of natural as well as synthetic antioxidants. Needless to say, in today's scenario, the use of synthetic antioxidants is diminishing due to increasing public awareness related to their long-term carcinogenic effect which has brought about strict legislation on their use as food additives. Nowadays, natural antioxidants are increasingly being preferred over their synthetic counterparts. Presently, the importance of the plant-based antioxidant constituents in providing protection against deadly diseases like cancer and heart problems as well as promoting overall health is increasingly being realized all over the world [3].

Phytochemicals derived from plants are major source of antioxidants. These phytochemicals are redox-active molecules and are dynamic to maintain redox balance in the body. Undoubtedly, plant-derived natural antioxidants are supposed to have more progressive effect on the body than synthetic ones. This is because plant constituents are a part of physiological functions of living flora and thus well suited to the human body. In recent years, the rising importance of biologically active components of plant origin has gained increased significance as highly promising prophylactic and restorative measures to combat diseases caused by oxidative stress. Higher plants, in particular, angiosperms, are used and explored as antioxidant sources. Cryptogams, especially bryophytes, hold rich reservoir of unique phytochemicals imparting them a strong defense mechanism to survive under highly diverse habitats despite having a non-lignified structure. There is huge potential to utilize this untrapped resource in modern healthcare as eco-friendly antibiotics and antioxidants [4].

Bryophytes, including liverworts, hornworts, and mosses, are phylogenetically placed between algae and vascular plants and form a unique division in the plant kingdom. They are small, mostly terrestrial, photosynthetic, spore-bearing plants that generally require a humid environment but can be found all over the world. These are represented by ca 7266–9000 liverworts, ca 221–225 hornworts, and 12,700–13,373 mosses [5, 6]. This large diversity of bryophytes also act as a "remarkable reservoir" of natural products or secondary compounds such as terpenoids, flavonoids, alkaloids, glycosides, saponins, anthraquinons, sterols, and other aromatic compounds. Many of them show interesting biological activity and become a potential source of different medicines. They also possess anticancer and antimicrobial activity due to their unique chemical constituents [7].

2. What are antioxidants?

The chemical reaction that can produce free radicals and leads to chain reactions that may damage the cells of organisms is known as the oxidation, and the compounds that inhibit or retard the oxidation of compounds are known as antioxidants. Antioxidants are broadly classified into three groups [8].

1. The first group of antioxidants is the enzymes which include catalase, superoxide dismutase, peroxidase, and glutathione reductase along with the minerals like Se, Cu, Zn, Fe, Mn, etc. that act as cofactors of these enzymes.

2. The second group of antioxidants includes glutathione, vitamin E (tocopherols), vitamin C, lipoic acid, albumin, carotenoids (vitamin A), phenolics, and flavonoids.

3. The third group of antioxidants includes a complex group of enzymes like DNA repair enzymes, transferases, lipases, proteases, methionine sulfoxide reductase, etc. which are used for repair of damaged DNA, damaged proteins, oxidized lipids, and peroxides [9].

The chemical compounds and reactions which are capable in generating potential toxic oxygen species/free radicals are referred to as "prooxidants." They attack macromolecules including proteins, DNA, and lipids and cause cellular or tissue damage. In a normal cell, due to the result of imbalance between reactive oxygen species (ROS) and antioxidant defenses, the oxidative stress is generated. It can result in serious cell damage if the stress is massive or prolonged. This leads to improper functioning which causes different pathogenic conditions like aging, carcinogenesis, cardiovascular dysfunction, neurodegenerative diseases, etc.

The reactive oxygen species (ROS) is generated during the different essential processes like photosynthesis, respiration, and stress responses. These ROS can lead to the disruption of the normal physiological and cellular functions and also the biomolecules of plasma membranes and cell walls [10, 11]. Interestingly, there are both ROS producing as well as ROS quencher systems operational in various organelles of cell. Low levels of ROS are beneficial sometimes acting as signaling molecules for stress tolerance by causing upregulation of the genes involved in the pathway of synthesis of stress enzymes/metabolites. High concentration of ROS is, however, deleterious and needs to be scavenged by either the intake of antioxidants or body's own endogenous antioxidants.

3. Antioxidant property in bryophytes

Bryophytes constitute a group of small plants which form essential components of terrestrial ecosystems. These are moisture-loving plants found mostly at the sites where water is readily available [12]. Although nowadays these plants are increasingly being focused for therapeutic research, the backbone of therapeutics, i.e., the chemistry of the group, is too limited covering less than 10% of the bryophytes [13]. Bryophytes possess good biological activities. The diverse activities of the bryophytes ranged from antimicrobial, cytotoxic, antitumor, cardiotonic, allergy causing, irritancy and tumor effecting, insect anti-feedant, molluscicidal, piscicidal, plant growth regulatory to superoxide anion radical release inhibition and 5-lipoxygenase, calmodulin, hyaluronidase, and cyclooxygenase inhibitions [14].

Among all the bryophytes, liverworts, being remarkable reservoir of natural products, are therapeutically used worldwide, especially in Indian and Chinese systems of medicine for the treatment of hepatitis and skin disorders [15–17]. Mosses, though more diverse than liverworts, are relatively lesser explored for medicinal utility. The secondary metabolites identified from mosses belong to terpenoids, flavonoids, and bibenzyls. They are also rich in other compounds such as fatty acids, acetophenols, etc. Their antimicrobial activity is related to the specific chemical composition, structural configuration of compounds, functional groups, as well as potential synergistic or antagonistic interactions between compounds [14].

Bryophytes produce a number of secondary metabolites that strengthen these delicate plants with strong antioxidative machinery to cope up with biotic and abiotic stresses [18, 19]. To compensate for the absence of any special morphological and anatomical defense mechanism, these plants have developed active molecular and chemical defenses for their protection. The antioxidant defenses provide protection to the cell membranes and cell organelles against oxidative damage. Under unfavorable conditions, reactive oxygen species react with important cell constituents, viz., proteins and lipids, causing disruption of cell structure ultimately leading to cell damage. Antioxidant enzymes protect cells against the oxidative stress induced by both internal and external unfavorable conditions. High level of these antioxidants present in liverworts and mosses can serve as a future source for medicinally and cosmetically significant compounds [20].

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 phenolics and flavonoid compounds responsible for free radical scavenging. 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 [21]. Similarly, a search for antioxidant enzymes in a moss, *Brachythecium velutinum*, and a liverwort, *M. polymorpha*, showed the role of an enzyme, ascorbate peroxidase, in the removal of hydrogen peroxide [22]. In another study, the extract of *Plagiochasma appendiculatum* showed significant antioxidant activity by inhibiting lipid peroxidation and increasing superoxide dismutase and catalase activity [23]. Reverse-phase high-pressure liquid chromatography reported the presence of various phenolic compounds such as caffeic, gallic, vanillic, chlorogenic, p-coumaric, 3-4 hydrozybenzoic, and salicylic acid in the moss *Sphagnum magellanicum* [24]. Other studies determined the presence of phenols, flavonoids, saponins, tannins, and glycosides in *M. polymorpha*. These studies also indicated anticancerous role of flavonoids extracted from cell suspension cultures of *M. linearis* against colon cancer cell lines [25, 26]. The biological characteristics of the terpenoids and aromatic compounds isolated from bryophytes also showed antibacterial and antifungal activities [27, 28]. Like other plants, antioxidant activity of bryophytes is influenced by several factors, viz., altitude, tissue type, and seasons [29]. The biochemical compounds responsible for antioxidant activity are also subject to quantitative and qualitative change in response to changes in these factors.

Bryophytes are traditionally used in the Chinese, Indian, and American societies for various medicinal purposes. However, the ethnomedicinal use of bryophytes needs to be scientifically investigated and validated for active principles in order to bridge the gap between traditional knowledge and pharmacology. For this, the active principle responsible for the specific activity may be identified and purified. The study on the antioxidant activities of the extracts of *Oxytegus tenuirostris*, *Eurhynchium striatum*, and *Rhynchostegium murale* showed that the climate is the most important ecological factor that determines the antioxidant property of the moss. Depending on these factors, antioxidant amounts in the species vary both within themselves and between species [30]. The study on the total free radical scavenging activity of *Eurhynchium striatulum* and *Homalothecium sericeum* showed that these have very strong free radical scavenging activity [31].

The alpine moss, *Sanionia uncinata*, produces some secondary metabolites that help the plant against the environmental stresses such as UV, drought, and high temperatures. *S. uncinata* shows good antioxidant activity, free radical scavenging activity, reducing power, superoxide radical scavenging activity, and ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] cation scavenging activity [32]. A study on the extracts of *Polytrichastrum alpinum* revealed that isolated compounds have two to sevenfold increased antioxidant activity than their extracts [33]. The reducing power of plant extracts was reported to be directly correlated with their antioxidant activity [34] and is based on the presence of reductones, which exert antioxidant activity by breaking the free radical chain and donating a hydrogen atom [35].

The remarkable nature of polyphenolics in terms of antioxidant potential has been identified to cure many lifestyle diseases [36]. Polyphenolic molecules contain one or many aromatic rings with hydroxyl groups. Generally, the antioxidant capacity of the phenolics is directly related with the number of free hydroxyls and conjugation of side chains with the aromatic rings [37]. The phytochemical studies on *Thuidium tamariscellum* showed the presence of significant level of terpenoids in the moss. High antioxidant property shown by the plant is reported to be mainly due to the presence of considerable amount of terpenoids [38].

Studies also revealed that the total flavonoid contents of liverworts were generally higher than those of mosses. Acrocarpous mosses had generally higher values of these compounds than that of pleurocarpous mosses. The total flavonoid contents of bryophytes growing at lower light levels were higher than those growing in full-sun. Likewise, total flavonoid contents of epiphytic bryophytes were highest, while those of aquatic bryophytes were the lowest. Species growing at low-latitudes had higher flavonoid content than those at high latitudes [39]. Studies also revealed that the antioxidant values of liverworts were closer to those of vascular plants. Guaiacol peroxidase and catalase activity of *P. appendiculatum* was found higher than *Pellia endivaefolia*, while superoxide dismutase, ascorbic acid, proline, glutathione, and total phenols were found higher in *P. endivaefolia* than *P. appendiculatum* [40].

Antioxidant and free radical scavenging activities are in the focus of attention of both medical practitioners and dieticians. Free radicals are supposed to play a key role in the pathogenesis of many diseases [41]. Oxidation processes may also decrease the stability of drugs and foods. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been recognized as fundamental components of stress signal cascades [42] under both abiotic and biotic stresses [43, 44]. Bryophytes occupy a special position among plants because the haploid gametophyte dominates their life cycle. Some species have been studied for their tolerances to drought and water stress (flooding) [45, 46] or high nitrogen concentrations [47]. Mosses are common in the vegetation of all continents, but they are still highly marginalized in traditional medicines. The plants which can respond and adapt to drought stress are certainly better equipped with complex and highly efficient antioxidative defense systems comprising of protective nonenzymatic as well as enzymatic mechanisms that efficiently scavenge ROS and prevent damaging effects of free radicals [48].

Some crude extracts of mosses contain hypnogenols, biflavonoids, dihydroflavonols, polycyclic aromatic hydrocarbons, and hydroxy flavonoids [49–51]. Flavonoids are synthesized by plants in response to the microbial infection. This action is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [49]. A large number of bryophytes are used as medicines in alternative medicine system. **Table 1** enlists certain medicinal bryophytes having significant antioxidant potential (selected on the basis of studied literature) available.

The screening for the antioxidant property by DPPH and ABTS assays revealed slightly higher antioxidant activity in ethyl acetate extract of *M. polymorpha* than ethanolic extract. Luteolin was an important antioxidant compound present in the

S. no.	Name of bryophyte	Antioxidant compounds	Reference
1	*Asterella angusta*	Asterelin A, asterelin B, 11-O- demethylmarcantin I, and dihydroptychantol adibenzofuran [bis(bibenzyl)]	[52]
2	*Atrichum undulatum, Polytrichum formosum*	Phenolics	[53]
3	*Bryum moravicum*	Phenolics	[54]
4	*Diplophyllum albicans*, *D. taxifolium*	Diplophylline	[55]
5	*Dumortiera hirsuta*	RiccardinD [macrocyclic bis(bibenzyl)]	[56]
6	*Dumortiera hirsuta*	Cell wall peroxidases and tyrosinases	[57]
7	*Frullania muscicola*	3-Hydroxy–4′- methoxylbibenzyl 7,4–dimethyl-apigenin	[58]
8	*Jungermannia subulata, Lophocolea heterophylla, Scapania parvitexta*	Subulatin	[59]
9	*Lunularia cruciata*	Flavonoids and sesquiterpenes	[60]
10	*Marchantia paleacea var. diptera*	Superoxide dismutase	[61]
11	*M. polymorpha*	Plagiochin E, riccardin H, marchantin E, neomarchantin A, marchantins A and B	[62]
12	*Mastigophora diclados*	Sesquiterpenoids	[63]
13	*Pallavicinia lyelli*	Ascorbate peroxidase	[64]
14	*Pallavicinia* sp. *Plagiochila* sp., *Plagiomnium* sp. and *Mnium* sp., *Riccardia* sp.	Bicyclohumulenone, plagiochiline A, plagiochilide, plagiochilal B, menthanemonoterpenoids, triterpenoidal saponins, riccardins A and B, sacullatal	[65]
15	*Philonotis* sp., *Rhodobryum giganteum*	Triterpenoidal saponins, p-hydroxycinnamic acid, 7–8-dihydroxycoumarin	[66]
16	*Plagiochasma appendiculatum*	Prevent lipid peroxidation and increase antioxidant enzymes	[23]
17	*Polytrichastrum alpinum*	Benzonaphthoxanthenones (Ohioensins F and G)	[33]
18	*R. roseum*	Prevents lipid peroxidation and augments antioxidants	[67]
19	*Plagiochila beddomei*	Phenolics	[68]
20	*Sanionia uncinata*	Antioxidant enzymes	[32]
21	*Sphagnum magellanicum*	Phenolics	[24]
22	*Thuidium tamariscellum*	Terpenoids	[38]
23	*T. tamariscinum and Platyhypnidium riparioides*	Phenolics	[20]

Table 1.
List of some bryophytes and their reported compounds showing antioxidant activity.

extract apart from other phenolics and bis(bibenzyls) [16]. Similarly, glutathione was observed as an important antioxidant compound in the terrestrial moss, *Pseudoscleropodium purum*, growing in industrial environments which can be used as a biomarker for pollution monitoring [69]. Besides the above listed plants, there are several other bryophytes which are having significant antioxidant potential [70–72]. All these bryophytes could be explored further for purification of the bioactive components for future applications.

4. Conclusion

Natural antioxidants form a promising alternative for synthetic antioxidants in food, cosmetic, and therapeutic industries. Easy availability, low cost, and lack of any harmful effects on the human body make natural antioxidants much sought after source of nutraceuticals. These antioxidants which are naturally present in many plant products, viz., fruits, vegetables, and spices, are remarkable reservoirs of radical quenchers. Increasing incidences of diseases vis à vis soaring pollution on the earth necessitates the use of natural therapeutic antioxidants as regular dietary supplements for providing better and efficient healthcare. Earlier, the focus of the world scientific community was on angiosperms as popular source of antioxidants. Nowadays, there is seen a paradigm shift of scientific focus from conventional and traditionally overexploited plant sources to nontraditional and nonconventional herbs. One such group of plants holding great potential can be desiccation-tolerant bryophytes that are usually considered not so useful plants by the layman community. Interestingly, due to storage of rich biomolecules, these desiccation-tolerant plants can also serve as an efficient source of many such antioxidants which could be used for novel drug discovery.

Author details

Dheeraj Gahtori[1] and Preeti Chaturvedi[2*]

1 Department of Botany, Government Post Graduate College, Uttarakhand, India

2 Department of Biological Sciences, G.B. Pant University of Agriculture and Technology, Uttarakhand, India

*Address all correspondence to: an_priti@yahoo.co.in

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