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Review article

SOME INDIAN BRYOPHYTES KNOWN FOR THEIR BIOLOGICALLY ACTIVE COMPOUNDS

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ABSTRACT : Bryophytes are known to produce a great range of biologically active compounds viz. terpenoids, aromatic compounds, and acetogenins. A lot of these constituents have typical odour, tanginess, and bitterness, and exhibit a fairly curious collection of bioactivities and medicinal properties. Chemical studies of the bryophytes were neglected for a long time in India. They are stockroom of naturally occurring materials. Many of these materials display substantial biological activity. Investigations are hindered commonly because of too little amounts of plant material. The resulting low yields of components are then generally insufficient to allow testing for biological activity. *In vitro* culture and suitable chemical synthesis on a preparative scale are now being undertaken to overcome this difficulty. In present review the bryophytes of Indian territory and their biologically active compounds have been highlighted which need to be used in sustainable manner.

Keywords : Bryophytes, lipophilic, terpenoids, acetogenins, cytotoxic.

INTRODUCTION

The bryophytes are placed taxonomically between algae and pteridophytes; there are about 24000 species in the world. They are distributed further into three classes, Musci (mosses, 14 000 species), Hepaticae (liverworts, 6000 species) and Anthocerotae (hornworts, 300 species). The Hepaticae contain cellular oil bodies which are easily extracted with organic solvents, while the other two classes do not. A number of bryophytes (in particular, mosses) have widely been used as medicinal plants in India, to cure burns, bruises, external wounds, etc. The mosses and liverworts are medicinal plants and are said to possess certain biological activity and effect (Garnier, et. al., 1969; Suire, 1972; Ding, 1982; Wu, 1982; Ando & Matsuo, 1984). Some bryophytes show characteristic fragrant odors and an intense hot and bitter or saccharine-like taste. Generally, bryophytes are not damaged by insects, snails, slugs, and other small animals. Furthermore, some liverworts cause intense allergenic contact dermatitis and allelopathy. It has been demonstrated that most of the Hepaticae contain mainly lipophilic mono-, sesqui-, and diterpenoids, aromatic compounds (bibenzyls, bis-bibenzyls, benzoates, cinnamates, long-chain alkyl phenols, naphthalenes, phthalides, isocoumarins), and acetogenins which constitute the oil bodies. The biological activities of liverworts are due to these substances. At present, over 400 new compounds have been isolated and their structures elucidated (Asakawa, 1982; 1984; 1990; 1993; 1995). The biological characteristics of the terpenoids and aromatic compounds isolated from the liverworts are: (1) characteristic scents; (2) pungency and bitterness; (3) allergenic contact dermatitis; (4) cytotoxic, anti-HIV, and DNA polymerase β inhibitory; (5) antimicrobial and antifungal activity; (6) insect antifeedant activity, mortality, and nematocidal activity; (7) superoxide anion radical release inhibitory activity; (8) 5-lipoxygenase, calmodulin, hyaluronidase, cyclooxygenase inhibitory activity, and nitric oxide (NO) production inhibitory activity; (9) piscicidal and plant growth inhibitory activity; (10) neurotrophic activity; (11) muscle relaxing activity; (12) cathepsins B and L inhibitory activity; (13) cardiotonic and vasopressin antagonist activity; (14) antiobesity activity; and (15) synthesis of bioactive compounds from liverwort constituents will be discussed.

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Medicinal bryophytes and their biological activity and effects (Lydwiczuk, 2008)

Musci: *Bryum argenteum* Antidotal, antipyretic, antirhinitic activity; for bacteriosis; all bryogeographical region of India. *Cratoneuron filicinum* For malum cordis (heart disease); Western Himalayas (Lal, 2005). *Fissidens laxitextus* Diuretic activity; for growth of hair, burns, and choloplania (jaundice, icterus); Eastern Himalayas (Lal, 2005). *Funaria hygrometrica* For hemostatis, pulmonary tuberculosis, vomitus cruentus (hematemesis), bruises, and athlete's foot dermatophytosis (dermatomycosis, dermomycosis); all bryogeographical region of India (Lal, 2005). *Leptodictyum riparium* Antipyretic; for choloplania and uropathy; Western Himalayas (Lal, 2005). *Mnium cuspidatum* For hematostasis and nosebleed; Western and Eastern Himalayas(Lal, 2005). *Oreas martiana* For anodyne (pain), hemostasis, external wounds, epilepsy, menorrhagia, and neurasthenia (nervosism, nervous exhaustion); Western and Eastern Himalayas (Lal, 2005). *Philonotis fontana* Antipyretic and antidotal activity; for adenopharyngitis; Western and Eastern Himalayas, South India (Lal, 2005). *Plagiopus oederi* As a sedative; for epilepsy, apoplexy, and cardiopathy; Western Himalayas (Lal, 2005). *Polytrichum* species Diuretic activity; for growth of hair; all bryogeographical region of India (Lal, 2005). *Rhodobryum giganteum* Antipyretic, diuretic, and antihypertensive; for sedation, neurasthenia, psychosis, cuts, cardiopathy, and expansion of heart blood vessels; Western and Eastern Himalayas, South India (Lal, 2005). *Rhodobryum roseum* As a sedative; for neurasthenia and cardiopathy; Western and Eastern Himalayas (Lal, 2005). *Taxiphyllum taxirameum* Antiphlogistic; for hemostasis and external wounds; Western and Eastern Himalayas, South India and Central India (Lal, 2005).

Hepaticae: *Conocephalum conicum* Antimicrobial, antifungal, antipyretic, antidotal activity; used to cure cuts, burns, scalds, fractures, swollen tissue, poisonous snake bites, and gallstones: Western and Eastern Himalayas (Parihar, et. al., 1994). *Frullania tamarisci* Antiseptic activity: Western and Eastern Himalayas, South India (Parihar, et. al., 1994). *Marchantia polymorpha* Antipyretic, antihepatic, antidotal, diuretic activity; used to cure cuts, fractures, poisonous snake bites, burns, scalds, and open wounds: all bryogeographical region of India (Parihar et al., 1994). *Reboulia hemisphaerica* for blotches, hemostasis, external wounds, and bruises: all bryogeographical region of India (Parihar, et. al., 1994).

Biological Activity: Liverworts release volatile terpenoids or simple aromatic compounds when trodden which are responsible for intense sweet-woody, intense turpentine, sweet-mossy, fungal-like, carrot-like, mushroomy, or seaweed-like scents (Asakawa, 1984; Ludwiczuk, 2008).

Characteristic odor of liverworts: *Asterella* species: skatole-like; *Cheilolejeunea imbricata*: Strong milky smell; *Conocephalum conicum*: Camphoraceous, strong mushroomy, and lactone-like; *Conocephalum japonicum*: Higher plant *Houttuynia cordata*-like; *Frullania tamarisci*: Oak moss-like; *Lophocolea heterophylla*: Strong and distinct mossy; *L. bidentata*: Strong and distinct mossy; *L. minor*: Strong moss-like; *Odontoschisma denudatum*: Civet, animal-like; *Plagiochila sciophila*: Sweet-mossy and woody; *Porella gracillima*: Woody-earthy; *Radula perrottetii*: Castor-like, animal-like; *Targionia hypophylla*: Sweet turpentine

Almost all liverworts that smell of mushrooms contain oct-1-en-3-ol and its acetate, which is generally more abundant than the free alcohol. A small thalloid unidentified liverwort, *Asterella* species emits an intense unpleasant odor which is due to skatole and composed of 20 % of the total extract. The strong milk-like fragrance of *Cheilolejeunea imbricata* is due to a mixture of (*R*)-dodec-2-en-1,5-olide and (*R*)-tetradec-2-en-1,5-olide (Asakawa, et. al., 1995). Bicyclohumulenone, isolated from *Plagiochila sciophila* as a crystal, possesses an aroma reminiscent of a variety of scents based on a strong woody note, resembling the odor of patchouli, vetiver, cedar wood, iris, moss, and carnations.

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Tamariscol from *F. tamarisci* subsp. *obscura*, possesses a remarkable aroma reminiscent of the woody and powdery green notes of mosses, hay, costus, violet leaf, and seaweeds. Both compounds are important in commerce. They are used as perfumes as such or as perfume components of the powdery floral-, oriental bouquet-, fantastic chypre-, fancy violet-, and white rose-types in various cosmetics. There are three chemo-types of *Conocephalum conicum*. Types 1, 2, and 3 emit (–)-sabinene, (+)-bornyl acetate, and methyl cinnamate as the major components, respectively, which are responsible for the characteristic odor of each type (Toyota, et. al., 1997a). The strong and distinct mossy odor of *Lophocolea heterophylla* and *L. bidentata* is due to a mixture of (–)-2-methylisoborneol and geosmin (Toyota, et. al., 1997b). The sweet turpentine-like odor of French *Targionia hypophyll*a is due to a mixture of *cis*- and *trans*-pinocarveyl acetates (Toyota, et. al., 1990).

Tanginess and bitterness

Some genera of the Hepaticae produce intense pungent and bitter substances which exhibit interesting biological activities described in subsequent sections. *Porella verni-cosa* complex contain potent pungent substances,

Allergenic contact dermatitis

Frullania species are notable as liverworts that cause very intense allergenic contact dermatitis (Asakawa, 2004). The allergy-inducing substances are sesquiterpene lactones, (+)-frullanolide and (–)-frullanolide, which have been isolated from *Frullania dilatata* and *F. tamarisci* subsp. *tamarisci*, respectively (Asakawa, 1982). Both dihydrofrullanolides with an α-methyl-γ-butyrolactone isolated from the above mentioned liverworts does not cause allergy. *F. inflata*, and the other *Frullania* species which contain sesquiterpenes with α-methylene-γ-butyrolactones cause strong allergenic contact dermatitis as does *Schistochila appendiculata*. The allergens of the latter are long-chain alkylphenols, 3-undecyl, 3-tridecyl, 3-pentadecyl, and 3-heptadecyl phenols, long-chain alkyl salicylic acids, 6-undecyl, 6-tridecyl, 6-pentadecyl salicylates, and their potassium salts, potassium 6-undecyl, 6-tridecyl, and 6-pentadecyl salicylates as well as 6-undecyl catechol (Asakawa, 1984). Such dermatitis is similar to that caused by the long-chain alkylphenols of the fruit of *Ginkgo biloba* and Anacardiaceae plants. *Marchantia polymorpha* and *Metzgeria furcata* also cause allergenic contact dermatitis but their allergens have not been isolated yet.

Cytotoxic, anti-HIV-1, and DNA polymerase inhibitory

A few eudesmanolides and germacranolides possessing inhibitory activity against KB cells have been isolated from liverworts. *C. conicum* and *Wiesnereilla denudata* contain guaianolides which exhibited cytotoxic activity against P-388 lymphocytic leukemia[7] . The crude ether extract (4–20 µg/ml) of the following liverworts showed cytotoxicity against P-388 *in vitro* (Toyota et al., 1990): *Lophocolea heterophylla*, *Pellia endiviifolia*, *Porella caespitans*, *P. perrottetiana*, and *Radula perrottetii.* On the other hand, *Frullania ericoides*, *F. muscicola*, *F. tamarisci* subsp. *obscura*, *Pallavicinia* sp., *Plagiochila sciophila*, and *Supraceanthus semirepandus*, were not active against P-388. 2,3-Secoaromadendrane-type sesquiterpenoids, plagiochiline A, plagiochiline A 13-octanoate, and 12-hydroxyplagiochiline A 13-2*E*,4*E*-dodecadienoate isolated from *Plagiochila* sp. showed cytotoxic activity (ID50 3, 0.05, 0.05 µg/ml, respectively) against P-388 (Huneck et al., 1986). Polygodial isolated from *P. vernicosa* complex, sacculatal from *Pellia endiviifolia*, and two 2,3-secoaromadendrane-type sesquiterpene hemiacetals, and plagiochiline A 13-decanoate from *P. ovalifolia* showed cytotoxic activity (2–4 µg/ml) against melanoma (Toyota & Asakawa, 1993).

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Riccardins A and B from *Riccardia multifida* inhibited KB cells at a concentration of 10 and 12 µg/ml, respectively. Many *Plagiochila* species and *R. perrottetii* contained cytotoxic plagiochiline A (0.28 µg/ml) and perrottetin E (12.5 µg/ml) against KB cell, respectively The thalloid liverwort, *M. polymorpha*, which can cause allergenic contact dermatitis, shows inhibitory activity against Gram-positive bacteria, and has diuretic activity. The methanol extract (100–150 g) of *M. polymorpha* was chromatographed on silica gel and Sephadex LH-20 to give cyclic bis-bibenzyls, marchantin A (MA) and its analogs (MB-G) (Toyota & Asakawa, 1993). The yield of MA is dependent upon *Marchantia* species. 80 to 120 g of pure MA has been isolated from 6.67 kg of dried *M. paleacea*. This thalloid liverwort elaborates not only the marchantin series, marchantin A, B, D, and E, but also the acyclic bis-bibenzyls, perrottetin F and paleatin B. Marchantins A, B, D, paleatin B, and perrottetin F show DNA polymelase β inhibitory (ID50 14.4–97.5 µM), cytotoxic (3.7–20 µM against KB cell), and anti-HIV-1 (5.30–23.7 µg/ml) activity [36b]. Marchantin A also shows cytotoxicity (T/C 117) against P-388[7]. *Blasia pusilla* produces bis(bibenzyl) dimers, pusilatins A–D. Pusilatins B and C possess DNA polymerase β inhibitory activity (IC50 13.0 and 5.16 µM), moderate cytotoxicity against KB cell (ED50 13.1 and 13.0 µg/ml), and weak HIV-RT inhibitory activity (Asakawa, et. al., 1988).

Antimicrobial and antifungal activity

Several liverworts, *Bazzania* species, *Conocephalum conicum*, *Dumortiera hirsuta*, *Marchantia polymorpha*, *M. furcata*, *Pellia endiviifolia*, *Plagiochila* species, *Porella vernicosa* complex, *P. platyphylla*, and *Radula* species show antimicrobial activity (Asakawa, 1984). Several such as *Bazzania* species, *C. conicum*, *Diplophyllum nanum*, *Lunularia cruciata*, *Marchantia polymorpha*, *Plagiochila* species, *Porella vernicosa* complex, and *Radula* species display antifungal activity (Asakawa, 1984). Marchantin A from many *Marchantia* species, *M. chenopoda*, *M. polymorpha*, *M. paleacea* var. *diptera*, *M. plicata*, and *M. tosana*, shows antibacterial activity against *Acinetobacter calcoaceticus* (MIC 6.25 µg/ml), *Alcaligenes faecalis* (100 µg/ml), *Bacillus cereus* (12.5 µg/ml), *B. megaterium* (25 µg/ml), *B. subtilis* (25 µg/ml), *Cryptococcus neoformans* (12.5 µg/ml), *Enterobacter cloacae*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonela typhimurium* (100 µg/ml), and *Staphylococcus aureus* (25 µg/ml) (Asakawa, 1984). They also have antifungal activity against *Alternaria* sp., *Aspergillus fumigatus* (MIC 100 µg/ml), *A. niger* (25-100 µg/ml), *Candiada albicans*, *Microsprorum gypseum*, *Penicillium chrysogenum* (100), *Piricularia oryzae* (12.5 µg/ml), *Rhizoctonia solani* (50 µg/ml), *Saccharomyces cerevisiae*, *Sporothrix schenckii* (100 µg/ml), and the dermatophytes *Trichophyton mentagrophytes* (3.13 µg/ml) and *T. rubrum* (100 µg/ml). Sacculatal, isolated from *Pellia endeviifolia* showed strong antibacterial activity against *Streptococcus mutans* (dental caries) at LD50 8 µg/ml, however, polygodial is less active (100 µg/ml) than sacculatal (Toyota, et. al., 1990).

Insect anti-feeding, mortality, and nematocidal activity

Plagiochiline A found in several *Plagiochila* species, is a strong antifeedant against the African army worm (*Spodoptera exempta*) (Asakawa, 1982). The compound shows nematocidal activity against *Caenorphabdiitis elegans* (111 µg/ml) (Asakawa, 1984). The pungent sacculatal kills tick species *Panonychus citri*. A series of natural drimanes and related synthetic compounds was tested for antifeedant activity against aphids (Tori et al, 1993). Natural (–)-polygodial and the synthetic (+)-enantiomer showed similar levels of activity as aphid antifeedants. (–)-Polygodial killed mosquito larvae at a concentration of 40 ppm and had mosquito repellent activity which is stronger than the commercially available DEET. Plagiochilide, isolated from *Plagiochila* species, killed *Nilaparvata lugens* (Delphacidae) at 100 µg/ml (Toyota, et. al., 1990).

Superoxide release inhibitory activity

Excess superoxide anion radical (O_2^-) in organisms causes various angiopathies, such as cardiac infarction, and arterial sclerosis. Infuscaic acid (clerod-3,13(16)-14-trien-17-oic acid) from *J. infusca* and plagiochilal B inhibit the release of superoxide from rabbit PMN at IC50 0.07 and 6.0 µg/ml, respectively and from guinea pig peritoneal macrophage induced by formyl methionyl leucyl phenylalanine (FMLP) at IC50 40 µg/ml, and 25.0 µg/ml respectively (Toyota & Asakawa, 1993).

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Norpinguisone methyl ether from *Porella* sp. exhibits 50 % inhibition of the release of superoxide from the guinea pig peritoneal macrophage at 35 µg/ml. The same activity (IC50 7.5 µg/ml) has been found in cyclomyltaylyl-3-caffeate from *Bazzania* sp. Other sesquiterpenoids, plagiochilide isolated from *Plagiochila fruticosa,* norpinguisone from *Porella vernicosa*, bicyclogermacrenal from *C. conicum*, herbertenediol and infuscaside A, and infuscaside B from *J. infusca*, and perrottetianal A from *Porella perrottetiana* also inhibit superoxide release from guinea pig peritoneal macrophage (IC50 12.5–50 µg/ml) (Asakawa, 1984). Radulanin K from *Radula javanica* inhibits the release of superoxide anion radical from guinea pig macrophage (IC50 6 µg/ml) (Toyota, et. al., 1990). Polygodial and sacculatal also show superoxide anion radical release inhibition at 4.0 µg/ml from guinea pig peritoneal macrophage (Toyota, et. al., 1990).

Piscicidal and plant growth inhibitory activity

The strongest piscicides are the pungent (–)-polygodial from *P. vernicosa* complex and sacculatal from *P. endiviifolia* and *P. levieri*. Killiefish (*Oryzia latipes*) is killed within 2 h by 0.4 ppm solution of and (Asakawa, 1982; 1984). Sacculatal and 1β-hydroxysacculatal also kill killie-fish within 20 min at 1 ppm [20]. Killie-fish is also killed within 2 h by a 0.4 ppm solution of synthetic pungent (+)-polygodial. Hence, piscicidal activity is not affected by the chirality of polygodial. Polygodial is also very toxic to fresh water bitterlings, which are killed within 3 min by a 0.4 ppm solution (Asakawa, 1984).

Neurotrophic activity

Plagiochilal B and plagiochilide from *Plagiochila fruticosa* show not only acceleration of neurite sprouting but also enhancement of choline acetyl transferase activity in a neuronal cell culture of the fetal rat cerebral hemisphere at 10–5 M (Asakawa, 1995; Toyota & Asakawa, 1993). Plagiochin A also shows the same activity at 10–6 M (Nagashima, et. al., 2004). Two bitter diterpene glucosides, infuscaside A and B, show neurite bundle formation at 10–7 M (Toyota, et. al., 1990).

Muscle relaxing activity

Marchantin A and the related cyclic bis-bibenzyls are structurally similar to bis-bibenzyl-isoquinoline alkaloids such as *d*-tubocurarine, which are pharmacologically important muscle relaxing active drugs. Amazingly, marchantin A and its trimethyl ether also show muscle relaxing activity (Bardón et al., 1999). Nicotine in Ringer solution effects maximum contraction of rectus abdominis in frogs (RAF) at a concentration of 10–6 M. After preincubation of marchantin A trimethyl ether (at a concentration of 2 × 10–7–2 × 10–4 M) in Ringer solution, nicotine (10–8–10–4 M) was added. At a concentration of 10–6 M, the contraction of RAF decreased by about 30 %. *d*-Tubocurarine exhibits similar effects as does with acetyl choline (Bardón et al., 1999). Although the mechanism of action of marchantin A and its methyl ether in effecting muscle relaxation is still unknown, it is interesting that these cyclic bis-bibenzyls possessing no nitrogen atoms, cause concentration-dependent decrease of contraction of RAF. Marchantin A and its trimethyl ether also had muscle relaxing activity in vivo in mice. MM2 calculations indicate that the conformation of marchantin A and its trimethyl ether and the presence of an *ortho* hydroxyl group in and an *ortho* methoxyl group in contribute to the muscle relaxing activity (Asakawa, 1993). Marchantin A triacetate and 7',8'-dehydromarchantin A and acyclic bis(bibenzyls), such as perrottetin E and F did not show any muscle relaxing activity.

Cardiotonic and vasopressin (VP) antagonist activity

Marchantin A shows cardiotonic activity [increase coronary blood flow (2.5 ml/min at 0.1 mg)]. Prenyl bibenzyl from *R. perrottetii* indicates vasopressin antagonist activity (ID50 27 µg/ml). However, 2-geranylbibenzyl from the same liverwort did not show VP antagonist activity (Asakawa, 1984).

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Liver X-receptor (LXR)_ agonist and (LXR)_ antagonist activity

Liver X receptors (LXR)α agonist and (LXR)β share considerable sequence homology and several functions, respond to the same endogenous and synthetic ligands and play critical roles in maintaining lipid homeotasis. Riccardin C and riccardin F, isolated from the liverwort *Reboulia hemisphaerica* function as an LXRα agonist/LXRβ antagonist and an LXRα antagonist, respectively (Tamehiro et al., 2004). Riccardin C effectively enhances cholesterol efflux from THP-1 cells. This compound may provide a novel tool for identifying subtype function and drug development against antiobesity.

Synthesis of bioactive compounds from liverwort constituents

The stem-leafy liverwort *Porella perrottetiana* elaborates a large amount of labdanediol. which is extremely expensive aroma originating from mammals. A highly oxygenated labdanes, for example, ptychantin A to folskolin and its congener, were found in the liverwort *Ptychantus striatus* belonging to the Lejeuneaceae as the major component (Asakawa, 2001).

CONCLUSION

Most of the compounds isolated from or detected in the bryophytes are lipophilic terpenoids (mono-,sesqui-, and diterpenoids) and aromatic compounds, of which only a few nitrogen- or sulfur-containing compounds have been found (Asakawa, 2001; Banerjee, 2001; Alam, et. al., 2011). It is remarkable that most of the sesqui- and diterpenoids found in liverworts are the enantiomers of those found in higher plants. Mono- and sesquiterpenoids are very rare in mosses and hornworts, but di- and triterpenoids have been isolated from certain mosses. At present, only 5 % of the total bryophytes have been studied chemically. The major contribution in this direction is came from the Japanese workers and this review is based on their reports but only those plants are included which are found in India. The aim of this to attracts the Indian workers. However, in India recently some work has been done on antimicrobial activities of liverworts like *Dumortiera hirsuta* and *Plagiochasma rupestre* (Alam, et. al., 2011; Alam, 2012) and interesting results have been observed, therefore more and more research on these aspects of bryophytes is a needed.

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