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RESEARCH ARTICLES

Gas chromatography-mass spectrometry (GC–MS) profiling of aqueous methanol fraction of *Plagiochasma appendiculatum* Lehm. & Lindenb. and *Sphagnum fimbriatum* Wilson for probable antiviral potential

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Abstract

The bryophytes consist of liverworts, mosses, and hornworts, among which the liverworts are quite different in having cellular oil bodies and contain numerous terpenoids, acetogenins, quinones, phenylpropanoids, flavonoids, etc. These metabolites exhibit interesting biological activity such as allergenic response, insecticide, cytotoxic, neurotrophic, antimicrobial, and anti-HIV actions, etc. Though several bioactive compounds have been isolated in many liverworts, yet most of the liverworts have been unexplored till date regarding their phytochemistry. The ability of liverworts to generate a wide range of important phytochemicals makes them a hoard of bioactive compounds. In the past, a few species of bryophytes have been evaluated against a few viruses and interesting results were obtained that showed their role as an immunity enhancer against viral infection. The phytoconstituents found in liverworts and mosses can be useful to increase human immunity against a variety of viruses, including SARS-CoV-2. Keeping this in view, one of the most developed and robust metabolomics technologies, Gas chromatography-mass spectroscopy (GC–MS) was used to estimate the various phytoconstituents found in a commonly growing thalloid liverwort, *Plagiochasma appendiculatum,* and moss *Sphagnum fimbriatum*. The obtained profiles were appraised for their bioactive potential and probable role as antiviral agents.

Keywords Antiviral · Bryophytes · GC–MS analysis · Liverworts · Moss · Secondary metabolites

Introduction

After angiosperms, bryophytes constitute the second largest diversity of green terrestrial plants (Asakawa 2007; Tedela et al. 2014). Bryophytes have three classes, viz., liverworts (Marchantiophyta), hornworts (Anthocerotophyta), and mosses (Bryophyta). Among these, liverworts are quite different from the other two classes in having oil bodies and a variety of bioactive secondary metabolites. Many liverworts have been utilized in medications to prevent and cure a variety of ailments all over the globe (Bodade et al. 2008; Sabovljevic et al. 2016). Bryophytes are thought to be a great repository of new natural products or secondary chemicals. Hundreds of novel phytochemicals have been isolated from bryophytes, especially liverworts, and determined chemically (Basile et al. 1999; Asakawa et al. 2013; Alam 2021). Some of these bioactive compounds have been found to have antimicrobial properties due to the presence of bi-flavonoids (Lopez-Saez 1996). Likewise, the existence of several phenolic compounds such as caffeic, gallic, 3–4 hydrobenzoic, vanillic, chlorogenic, p-coumaric, and salicylic acid was discovered using reverse phase high-pressure liquid chromatography considering the moss, *Sphagnum magellanicum* (Montenegro et al. 2009). Bryophytes are the chosen wildly growing plants to treat skin diseases and liver disorders (Friederich et al. 1999; Saroya 2011; Gokbulut et al. 2012). Though mosses, despite having a wider diversity of species, have been examined less thoroughly for therapeutic reasons than liverworts due to the natural absence of oil bodies, few of the species showed good amounts of terpenoids, bibenzyls, flavonoids, fatty acids, and acetophenols in them (Asakawa et al. 2013; Asakawa and Ludwiczuk 2018).

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A healthy resilient mechanism of human beings continuously defends the body from the occurrence of innumerable microorganisms, but when this system deteriorates, it results in many long-lasting diseases like SARS-CoV-2 which cannot be treated perfectly through conventional medicines. Many recent reports specify a close interface between SARS-CoV-2 and the immune response of an individual. This activated resistant reply due to SARS-CoV-2 in an infected individual can be affected in two stages; the preliminary incubation or non-severe and the following final severe stage (Shi et al. 2020). If the resistance of the infected individual is robust, then the advancement from the initial stage into the second stage will be delayed and help the system in the early elimination of the virus.

Hence, the confrontation-boosting approaches are very significant in providing immunity at the early stages of viral attacks. This depends upon the total health of the individual and its endogenous immune response to check the initial action of the virus (Shi et al. 2020). If the immunity of the infected individual is feeble, then the virus will flourish and replicate effortlessly, which results in immense harm to the body (Chen et al. 2020). It has been seen that SARS-CoV-2 affects respiration, kidney functions, liver metabolism, gastrointestinal tract, cardiac, and central nervous system (Huang et al. 2020; Liu et al. 2020). Thus, many deaths have happened due to multiple organ failures. Based on the information available about SARS-CoV-19, one thing is clear that the degree of immunity is the primary defense deciding death and life for someone afflicted with the virus (Fan et al. 2022). There has been no known occurrence of viruses infecting bryophytes, thus it's probable that their phytochemicals provide defense against viruses. Hillhouse (2003) showed that a bioflavonoid found in bryophytes can inhibit a wide range of viruses. Considering the resilient nature of bryophytes against viruses, the present research work has been done to compare the phytoconstituents profiles of the Uttarakhand populations of a liverwort, *Plagiochasma appendiculatum* Lehm. & Lindenb, and a moss *Sphagnum fimbriatum* Wilson and to envisage the possibility of these taxa to be evaluated for antiviral potential.

Material and method

Collection of plant and identification

The selected bryophytes were collected in December 2020, from Nainital, Uttarakhand (India) at an altitude of ca. 2084 msl. All samples were collected from their natural habitats in the afternoon to get optimum level of phytoconstituents. The random sampling approach was used during the collection within a stretch of 1 km. The identification was done based on available herbarium specimens and literature available at Bryotechnology Laboratory, Banasthali Vidyapith (Rajasthan), India. The taxonomic data of the reference specimens *Plagiochasma appendiculatum* Lehm. & Lindenb. (BURI-1138/2000) and *Sphagnum fimbriatum* Wilson (BURI-1145/2000) was deposited in the Banasthali University Rajasthan India Herbarium (BURI).

Preparation of plant material

The thalli were first precisely cleaned with tap water to remove soil particles and other plant debris then used deionized water for the final wash. Washed thalli were then transferred to liquid nitrogen (− 80 °C) and taken to the research laboratory, where the frozen thalli remained kept at the temperature of − 80 °C till further study. Before extract preparation, the thalli of both the species were air-dried at room temperature and pulverized into powder for extraction. The powder (5 g) was macerated in 80% methanol and hexane and allowed to stand for 48 h in an orbital shaker at 50 °C. The extract was filtered and stored at 4 °C until needed (Vats and Alam, 2013).

Gas chromatography-mass spectroscopy (GC–MS) analysis

For the GC–MS analysis, the technique described by Abu Bakar et al. (2015) was applied. The plant sample's crude extract was examined using a Thermo Scientific Triple Quadruple GC–MS (trace 1300, Tsq 8000 triple quadruple MS). The column temperature was initially set to 50 °C for 4 min, and then for 20 min, it was raised to 320 °C at a rate of 7 °C/min. The injector temperature was set to 280 °C (split mode, 20:1, injection volume = 0.1 µL). With a runtime of 60 min, the flow rate of the helium carrier gas was set at 1 mL/min. To acquire mass spectra in the range of m/z 40–700, electron ionization at a potential of 70 eV was utilized. The sample's chromatogram was determined by comparing the mass spectra to library data and the GC retention time to established standards.

Results and discussion

The GC–MS chromatograms for the methanolic extracts of gametophytes of selected liverwort and moss taxa were done (Figs. 1, 2). The chemical profiles have been attained for both the species and the percentage of each chemical compound was estimated from the relative peak area of each component in the chromatogram. The results show that the liverwort (*P. appendiculatum*) has 26 phytoconstituents (Table 1), while moss (*S. fimbriatum*) has only 13 diverse

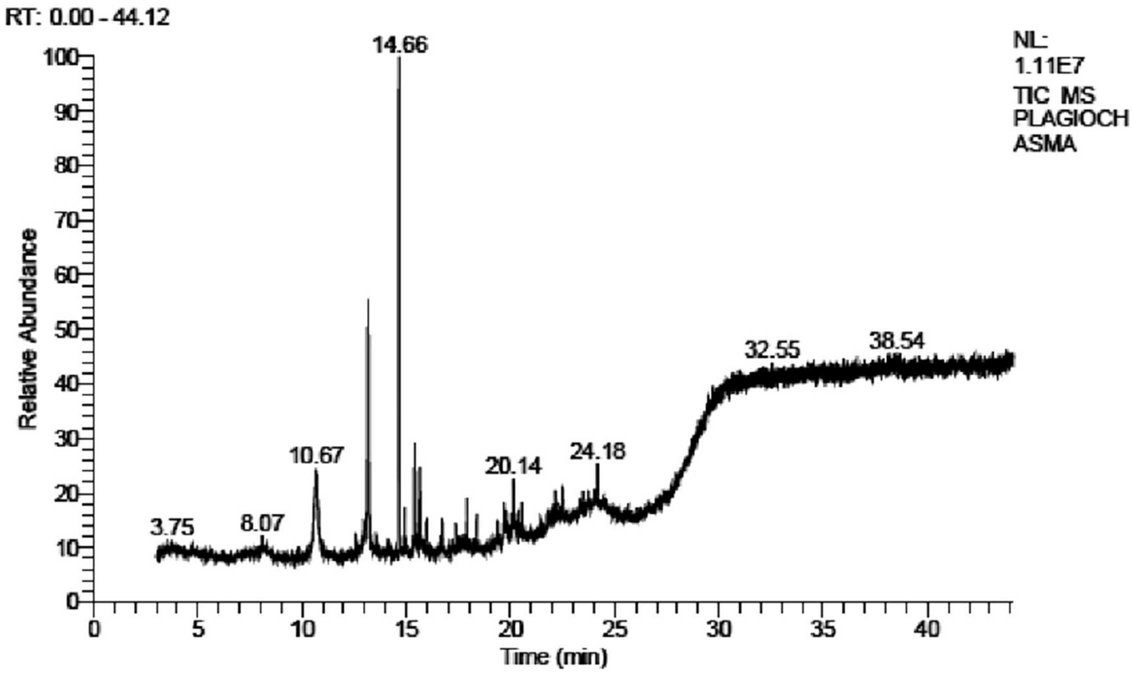

Fig. 1 GC–MS chromatogram of aqueous methanol fraction of *Plagiochasma appendiculatum*

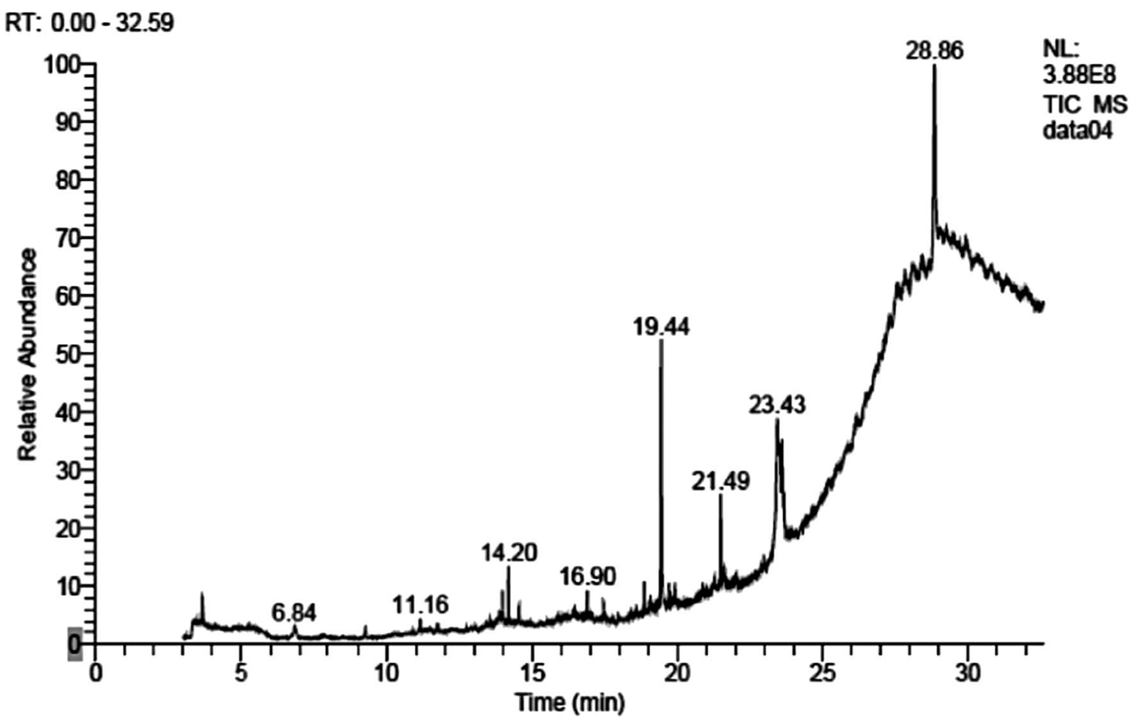

Fig. 2 GC–MS chromatogram of aqueous methanol fraction of *Sphagnum fimbriatum*

phytoconstituents (Table 2). Interestingly, the Caryophyllene and derivatives of phenol are present in both plants (Tables 1, 2).

In many previous studies, the chemical constituents identified in these plants have been found responsible for various medicinal activities. For instance, Caryophyllene

Table 1 Bioactive compounds found in aqueous methanol fraction of *Plagiochasma appendiculatum*

S. no.	RT (min)	Peak area (%)	Name of the compound	Molecular formula
1	10.67	15.74	2,6-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$
2	14.66	23.09	Caryophyllene	$C_{15}H_{24}$
3	14.91	2.99	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro- Aromandendrene à-acorenol á-Longipinene	$C_{22}H_{40}O_2$ $C_{15}H_{24}$ $C_{15}H_{26}O$ $C_{15}H_{24}$
4	15.66	5.96	2,4-Di-tert-butylphenol Phenol, 3,5-bis(1,1-dimethylethyl)- Phenol, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$ $C_{14}H_{22}O$ $C_{14}H_{22}O$
5	17.91	3.05	Octadecane, 3-ethyl-5-(2-ethylbutyl)- Ethanol, 2-(octadecyloxy)- Tetrapentacontane, 1,54-dibromo-	$C_{26}H_{54}$ $C_{20}H_{42}O_2$ $C_{54}H_{108}Br_2$
6	19.66	4.35	Phthalic acid, butyl undecyl ester Phthalic acid, isobutyl octadecyl ester 1,2-Benzenedicarboxylic acid, butyl octyl ester Phthalic acid, butyl tetradecyl ester Phthalic acid, 2-cyclohexylethyl isobutyl ester	$C_{23}H_{36}O_4$ $C_{30}H_{50}O_4$ $C_{20}H_{30}O_4$ $C_{26}H_{42}O_4$ $C_{20}H_{28}O_4$
7	19.80	2.86	tert-Hexadecanethiol 2-Hexadecanol 2-Nonadecanone 2,4-dinitrophenylhydrazine Oleic acid, 3-(octadecyloxy)propyl Ester Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{16}H_{34}S$ $C_{16}H_{34}O$ $C_{25}H_{42}N_4O_4$ $C_{39}H_{76}O_3$ $C_{26}H_{54}$
8	20.14	4.01	Octadecane, 3-ethyl-5-(2-ethylbutyl)- Heptacosane Tetradecane, 2,6,10-trimethyl- Tetrapentacontane, 1,54-dibromo-	$C_{26}H_{54}$ $C_{27}H_{56}$ $C_{17}H_{36}$ $C_{54}H_{108}Br_2$

Table 2 Bioactive compounds found in aqueous methanol fraction of *Sphagnum fimbriatum*

S. no.	RT (min)	Peak area (%)	Name of the compound	Molecular formula
1	6.84	2.43	Acetic acid methyl ester	$C_3H_6O_2$
2	11.16	20.50	Caryophyllene	$C_{15}H_{24}$
3	14.20	5.19	3,7,11,15- Tetra methyl- 2- hexadecen-1-ol	$C_{20}H_{40}O$
4	16.90	10.15	Phytol	$C_{20}H_{20}O$
5	19.44	18.28	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
6	21.49	6.08	Heptadecanoic acid 16- methyl- methyl ester Heptadecanoic acid 10- methyl- methyl ester Methyl stearate Hexacosane	$C_{19}H_{38}O_2$ $C_{19}H_{38}O_2$ $C_{19}H_{38}O_2$ $C_{26}H_{54}$
7	23.43	18.37	Propanoic acid Phenol 2,4- bis (1,1-di methyl ethyl)- phosphate	$C_3H_6O_2$ $C_{42}H_{63}O_3P$
8	26.16	4.72	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$
9	27.54	5.80	Dasycarpidan 1-methanol, acetate(ester)	$C_{20}H_{26}N_2O_2$

has biological activities, viz., antiviral, anti-inflammatory, anesthetic, anti-carcinogenic, anti-microbial, anti-tumor, analgesic, antibacterial, cytotoxicity, and anti-fungal activities (Selvaraju et al. 2021). The compound Phytol is reported to have antiviral, antimicrobial, anticancer, and anti-inflammatory properties (Subin et al. 2021). Hexacosane, Octadecane, Heptacosane essential oil showed a significant antiviral effect against Coxsackie virus B4 (Bouazzi et al. 2018). The methyl ester of hexadecanoic acid has antifungal and antibacterial properties (Mehdi et al. 2021). Oleic acid has a moderate antiviral effect on Parainfluenza virus type-3 (Sener et al. 2007). Recent investigations have proved that tert-Hexadecanethiol has antioxidant activity (Qanash et al. 2022). 2,4-di-tert-butylphenol, was shown to have a protective effect against Ab1-24 by decreasing neuronal cell damage (Choi et al. 2013). Aromandendrene could be considered a novel natural molecule for the possible development of appropriate SARS-CoV-2 drug candidates (Muhammad et al. 2020).

The Phthalic acid ester derivatives showed antiviral activity against dengue virus, human parainfluenza virus and chikungunya (Uddin et al. 2013). Tetrapentacontane, 1,54-dibromo- can treat chronic illnesses (Bensaad et al. 2022).

Though bryophytes are cosmopolitan in distribution, they are lesser explored for their phytochemistry compared to angiosperms. This reluctance was mainly due to their small size, low biomass and understandable difficulties in their collection and identification, hence, very few bryophytes have been evaluated for their phytochemistry till date. The achieved information through their GC–MS profiling exhibit that a range of vital phytochemicals is present in studied plants. These phytoconstituents are well known for their antioxidant, antimicrobial, cytotoxicity, antiviral activities, etc. Among the reported phytoconstituents, many of them have been validated to have antiviral activity against (Russo et al. 2020). This shows that the naturally present phytoconstituents in these plants have great potential to boost the immunity of human beings against a range of viruses, including SARS-CoV-2.

On comparison between the selected liverwort and moss taxa, it is evident the liverwort (*P. appendiculatum*) is richer in terms of phytochemistry than the moss (*S. fimbriatum*). The phytoconstituents are largely different between them except the Caryophyllene and a few derivatives of phenol that are common in both. This indicates that liverworts have more applicability in herbal remedies of the future. Nevertheless, the phytochemical profiles of both these bryophytes are quite rich, hence, they can provide useful bioactivities, viz., antimicrobial, antitumor, cytotoxic, cardioprotective, allergy triggering, etc., and these bryophytes also have antiviral potential based on earlier reports however further studies are required in this direction.

Conclusion

The GC – MS analysis indicates that both the bryophytes have useful phytoconstituents with vital bioactivities, including antiviral potential. Though the reported phytoconstituents haven't been estimated against SARS-CoV-2 yet, in the future there is a possibility that these might be useful in various medication strategies against the viruses. Until now, only 5% of the total bryophytes have been chemically investigated and the huge diversity of this group is still unexplored for medicinal practicality including antiviral properties.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest relevant to this article.

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