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Seasonal phytochemical screening and Gas Chromatography-Mass Spectroscopy analysis of *Timmia megapolitana* Hedw. (*Timmiaceae*) from Kumaun Himalayas, India

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ABSTRACT

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The present study was undertaken to evaluate the effect of seasonal variations on the phytochemical content of a moss species *Timmia megapolitana* Hedw. in its natural habitat. The preliminary phytochemical screening of *T. megapolitana* revealed the presence of alkaloids, flavonoids, terpenoids, phytosterols, and anthocyanins in methanolic extract whereas the GC-MS analysis further revealed the presence of nine bioactive compounds including methyl ester, methyl stearate, hexacosane, n-hexadecanoic acid, phytol, caryophyllene, heptadecane, boric acid trimethyl ester, phosphinic acid and pyridine. The periodic phytochemical profiling of aqueous methanol fraction of *T. megapolitana* revealed that the presence of bioactive compounds was more in summer season than the monsoon and winter season due to ambient abiotic stress factors. Hence, the findings suggest that the studied moss is a reliable and potential source of bioactive compounds. The quantity is greatly influenced by the seasonal variations hence and the required production of these secondary metabolites is also possible *in vitro* by the induction of related stress factor.

Keywords: Bryophytes, GC-MS, methanol extract, phytochemical, stress, *Timmia megapolitana*.

INTRODUCTION

Plants are of great importance in the field of pharmacology as most pharmaceutical industries depend on medicinal plants and their products as raw materials of many produce. Mostly, the tracheophytes are the preferred choice for this, but atracheate plants like bryophytes posses a variety of bioactive chemicals in the form of secondary metabolites that can be widely used as therapeutic herbs. Bryophytes constitute the second most prevalent plant group after angiosperms on this planet (Rice 2009). In India according to ENVIS-BSI (2021), nearly 2562 taxa of bryophytes, including 887 liverworts, 1636 mosses, 39 hornworts are present. These miniature plants have a huge wealth of phytochemicals in them, although it is a matter of fact that most Indian bryologists have mentioned various ecological and horticultural roles of bryophytes, but their phytochemicals have been ignored to a great extent (Frahm 2004). Mostly the Indian bryologists focus on morpho-taxonomy and diversity related studies on bryophytes. Thus, there is dearth of phytochemical-based studies on bryophytes in India.

Bryophytes have a tremendous ability to survive against a variety of biotic and abiotic stresses due to their peculiar and very simple resurrective gametophytes and sporophytes (Vitt et al. 2014) and for this reason their phytochemical constituents also differ from other large plants (Glime 2017).

In the recent past, various researchers from Japan, China and some other countries have done a lot of work in terms of identification of phytochemicals in bryophytes (Asakawa et al. 2013).

Though these secondary metabolites are produced as defense ploy for the host plant, yet they have medicinal significance for human beings. As a result, several bryophytes have been used as medicine for millennia, and numerous researches have been done to investigate their therapeutic characteristics (Nandy & Dey 2020).

The seasonal fluctuations in temperature, light, humidity, water availability have substantial effect on the metabolism of any plant species. Higher plants have a lot of research on these effects, but such studies regarding bryophytes are somewhat scanty though being the first land plants they are usually strongly influenced by abiotic pressures. They are also known to produce reactive oxygen species and other defense compounds under stress condition (Yadav et al. 2021).

Plant-based physiologically active compounds in their natural condition are referred to as phytochemicals. Phytochemicals are regarded to be effective in disease prevention because of their antioxidant capabilities (Karim et al. 2014).

Flavonoids have been shown to have health-promoting properties (Akinmoladun et al. 2007). Terpenoids are being researched for antibacterial, anticancer, and other pharmacological characteristics and they are used in traditional herbal remedies. Terpenoids appear to be common in bryophytes (Asakawa et al. 1981). So far, 15 distinct sterols have been discovered in bryophytes. Sitosterol and stigmasterol are found in all of the taxa investigated. Campesterol is more abundant in liverworts, whereas the rest of the sterols are found in mosses (Yoshinori et al. 1980). The phytochemicals formed in bryophytes provide a much needed defense not only against the abiotic stresses but also the biotic stresses such as microbial and insect attacks. In this way these biochemical components compensate the absence of anatomical and morphological adaptation, *viz*., thick cuticle and bark in bryophytes (Alam 2012).

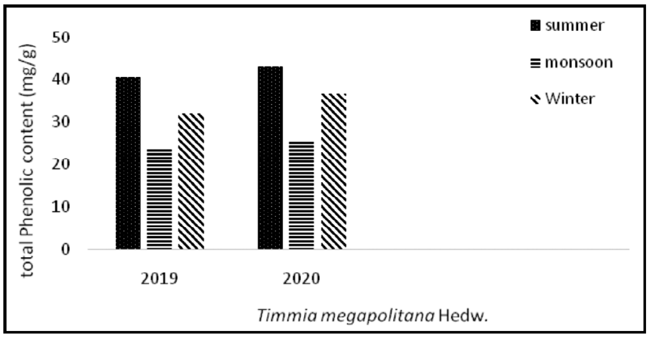

Figure 1.Total phenolic content of *Timmia megapolitana* in the year 2019 and 2020.

Bryophytes retain various phytochemicals in their thalli, for example flavonoids, terpenoids, sitosterol, stigmasterol, campesterol types of antioxidant molecules are usually present in gametophytes (Deora & Deora 2018). Cardiac glycosides are drugs that treat heart failure and arrhythmia. As secondary metabolites, several bryophytes synthesize these glycosides.

The quantity of these phytochemicals is always dependent upon the ambient environmental conditions both at micro and macro levels. Many of these have been identified by various research groups worldwide (Sabovljevic et al. 2017). The taxa that are known for their medicinal properties have been studied extensively to validate the claims about their medicinal values. For instance, moss genera such as *Philonotis*, *Bryum*, and *Mnium* have been mashed into a paste and administered as a poultice by the Chinese and Native Americans. In the Himalayan regions of India, burned moss ash mixed with fat and honey is used as an ointment for cuts, burns, and wounds. *Marchantia polymorpha*, a liverwort, is also used to treat boils and abscesses, may be because the immature archegoniophore looks like a boil when it initially emerges from the thallus (Muhammad et al. 2018). An extract of *Rhodobryum giganteum* has been demonstrated to enhance aorta blood transit by up to 30% in rats. For the treatment of 'haemophilia' B, transgenic *Physcomitrella* is now

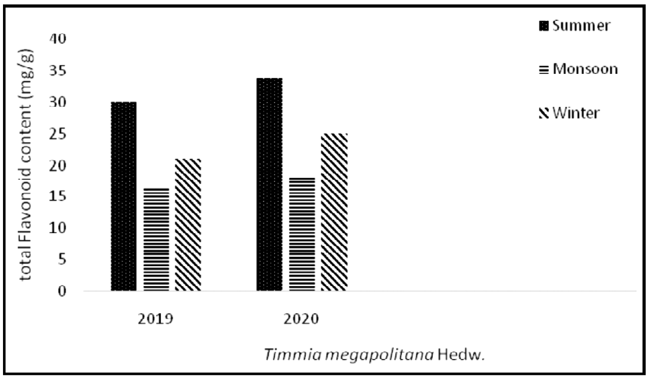

Figure 2. Total flavonoid content of *Timmia megapolitana* in the year 2019 and 2020.

being employed to create 'blood-clotting factor IX.' Calcined peat preparations have long been recognized as efficient and inexpensive germicides, and peat water has astringent and antibacterial effects (Chavhan 2017). 'Sphagnol,' a Peat Tar distillate, is excellent for eczema, psoriasis, pruritus, haemorrhoids, chilblains, scabies, acne, and other skin ailments, as well as for reducing the irritation caused by insect bites (Saxena 2004). The presence of biologically active chemicals is one of the characteristics that enable bryophytes to survive and maintain their place in today's flora (Sabovljevic et al. 2011).

Therefore, the present study has been done to find out the phytochemical profile of *T. megapolitana,* a commonly growing moss in Uttarakhand state of India. Not only the phytochemical profiling is done but also a quantitative comparison is made to evaluate the impact of different seasons regarding phytochemicals. Since the selected moss taxon (*T. megapolitana*) has a year-round occurrence in the studied area therefore it was assumed that it should have some peculiar phytochemicals that help in its sustenance.

The phytochemistry and biological activities of *T. megapolitana* was yet to be reported till date. Consequently, the purpose of this work was to evaluate the phytochemical contents and their existence through GC-MS analysis of *T. megapolitana*.

MATERIAL AND METHODS

Plant collection and identification: The samples of the selected moss (*T. megapolitana*) were collected in the month of May- June (summer), August-September (monsoon) and December - January (winter) in year 2019 and 2020, from the Nainital region of Uttarakhand (India) at an altitude of ca. 2084 msl. The moss samples were collected from their natural habitat in-between 11:20 am and 1:30 pm (IST) during this the average summer temperature range was between the highs of 23°C and lows of 21°C, in monsoon this range was between the highs of 22 °C and lows of 20 °C while in winter this range was between the highs of 10 °C and lows of 8°C. The random sample approach was used during the collection within a stretch of 1 km. The identification was done based on available herbarium specimens and literature at Bryotechnology laboratory, Banasthali Vidyapith (Rajasthan), India. The taxonomic data of the reference specimens (BURI-1395/2022) was accordingly submitted in the Banasthali University Rajasthan India Herbarium.

Preparation of plant material: The collected moss samples were first meticulously cleaned of soil and other plant detritus using clean water. Then transferred in liquid nitrogen and taken to the research laboratory, where the freeze plants were kept at the temperature of -80°C till further study. Prior to extract preparation of moss samples were air-dried at room temperature and pulverized into powder for extraction. The powder (5g) was macerated in 80% methanol and hexane and allowed to stand for 48h in an orbital shaker at 50°C. The extract was filtered and stored at 4°C until needed (Vats & Alam 2013).

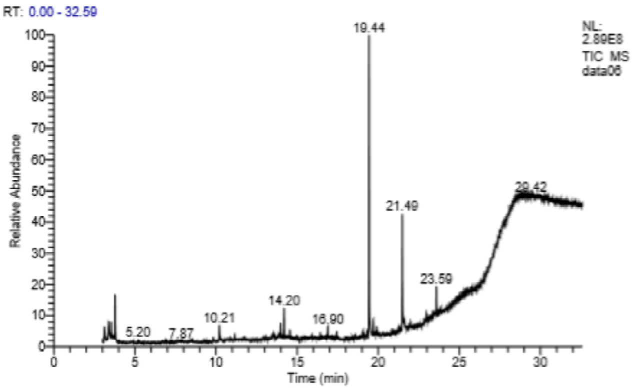

Figure 3. Gas Chromatography-Mass Spectroscopy chromatogram of aqueous methanol fraction of *Timmia megapolitana*.

Preliminary phytochemical screening: Phytochemical profiling of crude extract and aqueous methanol fraction of *T. megapolitana* was carried out using the procedure as described by Bhadauriya et al. (2018).

Determination of Total Phenolic Content: Colorimetrically, total phenolics were measured using the Folin- Ciocalteaeu technique (Vats et al. 2012). A test tube was filled with 0.5 ml of water and 0.125 ml of the methanolic extract. Folin-Ciocalteu reagent (0.125 ml), sodium carbonate solution (1.25 ml), and water (3 ml) were added in that order and left to stand for 90 minutes. At 760 nm, the absorbance was measured. Total phenol concentration was measured in gallic acid equivalents (GAE) based on dry material (mg GAE/g dry weight of sample). The mean and standard deviation are used to express the data.

Determination of Total Flavonoids Content: Total flavonoids content was estimated using the method of Zhishen et al. (1999). One ml extract was combined with 4 ml distilled water and 0.3 ml sodium nitrite solution at a concentration of 5% sodium nitrite. After 5 minutes, 0.6 ml of 10% aluminium chloride was added to the mixture and left to sit for 6 minutes before adding 2 ml of 1M sodium hydroxide. The interaction of flavonoid with sodium nitrite and aluminium chloride results in a colored flavonoid aluminium complex, which was detected at 510 nm using a spectrophotometer. The test samples were conducted with quercetin standards at concentrations ranging from 0 to100 μg/mL. The results were measured in milligrams of quercetin equivalent in 1 g of dried sample (mg QE/g).

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS) ANALYSIS

The method provided by Abu Bakar et al. (2015) was used for the GC-MS analysis. Crude extract of the plant sample was analyzed with Thermo Scientific Triple quadruple GC-MS (trace 1300, Tsq 8000 triple quadruple MS). The column temperature was set to 50°C for 4 minutes before being increased to 320°C at a rate of 7°C/min for 20 minutes. The injector temperature was set to 280°C (split mode, 20:1, injection volume = 0.1 μL). The helium carrier gas flow rate was set to 1 mL/min, with a total run time of 60 minutes. The electron ionization at 70 eV was used to obtain mass spectra in the region of m/z 40 to 700. By comparing the sample's mass spectra to library data and the GC retention time to known standards, the sample's chromatogram was identified.

Statistical Analysis: The results are shown as means (n = 3) of triplicates. The IBM SPSS Statistics 20 software was used to analyze all the data collected. The chosen variable was subjected to three-way interactions. Turkey's $p < 0.05$ post-test was used to compare the variance of data for each output variable multiple-comparison. All data is presented as a mean with standard error.

RESULTS

It is evident that phytoconstituents show a shift in their concentrations by over-expressing the defense responsive genes to fight during the stress (Isah 2019). Thus, in this study the gametophytes of *T. megapolitana* were analyzed with respect to their phytochemical contents over a time period of a year considering summer, monsoon and winter seasons as several of abiotic stress. The Gas chromatography-Mass Spectroscopy (GC-MS) was also used for the separation and investigation of multi-component mixtures for instance, essential oils, hydrocarbons, and solvents. The quantitative determination of trace compounds was done using the flame ionization detector and the electron capture detector.

Qualitative and quantitative analysis: The findings of a qualitative phytochemical investigation of *T. megapolitana* demonstrated high quantities of alkaloids, flavonoids, terpenoids, phytosterols, and anthocyanins in methanolic extracts. While alkaloids, flavonoids, phytosterols, and anthocyanins were absent in the hexane extract (Table 1). Finally, the methanol extract of the moss produced the best results. The total phenols and flavonoids content of *T. megapolitana* are expressed as gallic acid and quercetin, respectively (Tables 2, 3).

Table 1. Qualitative analysis of a methanolic and hexane extract of *Timmia megapolitana*. ++ (highly present), + (moderately present), - (absent)

Sl. No.	Phyto-constituents	Tests	In methanol	In hexane
1.	Alkaloids	Mayer's test	++	-
		Wagner's test	++	-
2.	Flavonoids	Ferric chloride test	++	-
		Lead acetate test	-	-
3.	Carbohydrate	Benedict test	-	-
4.	Terpenoids	Copper acetate test	++	-
5.	Phytosterols	Salkowaki test	++	-
		Lebermann-Burchard test	-	-
6.	Saponins	Froth test	-	-
7.	Phenols	Lead acetate test	+	-
8.	Tannins	Ferric chloride test	-	-
		Lead acetate test	-	-
9.	Anthocyanins	Concentrated H_2so_4	++	-

Table 2. Quantitative analysis of *Timmia megapolitana* in different seasons of the year 2019. (Data are the means and standard deviation of the mean for n = 3 independent experiments)

Variables	May - June (Summer)	August–September (Monsoon)	December–January (Winter)
Total Phenolic content (mg/g GAE)	40.32 ± 0.33^c	23.82 ± 0.19^b	32.02 ± 0.29^c
Total Flavonoid content (mg/g QE)	30.02 ± 0.27^c	16.25 ± 0.12^a	21.05 ± 0.19^b

Table 3. Quantitative analysis of *Timmia megapolitana* in different seasons of the year 2020. (Data are the means and standard deviation of the mean for n = 3 independent experiments)

Variables	May-June (Summer)	August–September (Monsoon)	December–January (Winter)
Total Phenolic content (mg/g GAE)	43.06 ± 0.35^c	25.32 ± 0.20^b	36.72 ± 0.30^b
Total Flavonoid content (mg/g QE)	33.76 ± 0.29^c	18.32 ± 0.13^a	25.02 ± 0.20^b

Gas Chromatography-Mass Spectroscopy profiling of aqueous methanol fraction of *Timmia megapolitana*: The GC-MS analysis of *T. megapolitana* methanolic extract revealed the presence of nine different chemicals. The chromatogram is presented in fig. 3, while the chemical constituents with their retention time (RT), concentration (%) and molecular formula are presented in Table 4. The bioactive components in the sample extract had a retention period ranging from 3.13 to 23.59 minutes. The following bioactive compounds were present in the GC-MS analysis carried on methanol fraction of *Timmia megapolitana*: Boric acid, tri-methyl -ester Phosphinic acid [(methyl (formyl) amino] methyl (2-Phenylethyl), Thiodiglycol 5- Benzyloxypyrimidine- 2-carboxylic acid, Pyridine, 2,4-Pentadienenitrile, Caryophyllene, Eicosane, 2-methyl- Heptadecane Octadecane 1,1'-[1-methyl-1,2-ethanediyl) bis (oxy)] bis- , 2,4Di- tert-butylphenol Phenol, 3,5-bis (1, 1-di methylethyl)-n-hexadecanoic acid, Hexadecanoic acid, methyl Ester, Phytol, Methyl stearate, Hexacosane.

DISCUSSION

Cryptogams such as bryophytes are relatively less explored in terms of their biological activities in relation to the climatic changes. However, as a fundamental element the environmental variability has a profound impact on all aspects of existence for any species that live under the changing atmospheric conditions. Bryophytes also shift their metabolism as per their need to sustain (Saini et al. 2022). Considering this the effect of seasonal variations on phytochemical profile of *T. megapolitana* has been estimated over its three distinct growing seasons from year 2019 to 2020 in this study. The season-wise observations and estimation has been summarized and discussed here.

Summer Season (May-June): At the sample collection site (Nainital, India), summer season is warm with average temperature range of 22.1 - 22.8^0 C. This region captures environmental abiotic stress, as well as the intensity and duration of photoperiods, during the summer season. In light of these circumstances, the results of this research revealed that total phenol and flavonoid levels were at an all-time high throughout this season (Lunic et al. 2022).

Monsoon Season (August-September): It is a moderately hot, rainy season with temperature in the average range of 21.3 – 20.3 ^{0}C. During monsoon, the

Table 4: Bioactive compounds found in aqueous methanol fraction of *Timmia megapolitana*

Sl. No.	RT (min)	Peak Area (%)	Name of the compound	Molecular formula
1.	3.13	4.13	Boric acid, trimethyl ester	$C_3H_9BO_3$
			Phosphinic acid, [(methyl) (formyl) amino] methyl (2-Phenylethyl)-	$C_{11}H_{16}N_{O3}P$
2.	3.38	5.56	Thiodiglycol 5- Benzyloxypyrimidine-2-carboxylic acid	$C_4H_{10}O_2SC_{12}H_{10}N_2O_3$
3.	3.78	6.87	Pyridine	C_5H_5N
			2, 4-Pentadienenitrile	C_5H_5N
4.	10.21	10.71	Caryophyllene	$C_{15}H_{24}$
5.	13.99	3.01	Eicosane, 2-methyl-	$C_{21}H_{44}$
			Heptadecane	$C_{27}H_{56}$
			Octadecane 1, 1'-[1-methyl-1,2-ethanediyl) bis (oxy)] bis-	$C_{39}H_{80}O_2$
6.	14.20	4.28	2,4 Di- tert-butylphenol	$C_{14}H_{22}O$
			3,5-bis(1, 1-di methylethyl)-	$C_{14}H_{22}O$
			n-Hexadecanoic acid	$C_{16}H_{32}O_2$
7.	19.44	45.70	Hexadecanoic acid, methyl Ester	$C_{17}H_{34}O_2$
8.	21.49	19.06	Phytol	$C_{20}H_{40}O$
			Methyl stearate	$C_{19}H_{38}O_2$
9.	23.59	23.59	Hexacosane	$C_{26}H_{54}$

collection site receives a heavy amount of precipitation as well as thunderstorms which form due to intensive atmospheric vortices and connective processes (Yamane & Hayashi 2006). The moisture for these thunderstorms comes from intense localized evaporation from open water bodies like rivers and lakes. Precipitation and humidity levels are higher throughout this season, allowing *T. megapolitana* to flourish well. In this climate, the moss reaches its maximum vegetative growth, and asexual or vegetative reproduction due to optimum metabolism (Thakur & Kapila 2017). Accordingly, the findings of this investigation revealed that the lower phenol and flavonoid contents in this season compared to summer.

Winter Season (December-January): Fruiting season in Nainital is very cool, dry where temperature varies from an average range of 10.6 – 8.8° C. Temperatures decrease dramatically throughout this season, making the environment extremely harsh and devoid of nutrition (Peters et al. 2019). During this phase, *T. megapolitana* completes sexual reproduction and begins sporophyte (fruiting body) development. The formation of a sporophyte, a diploid and spore-producing structure, signifies the end of gametophytic phase of life cycle. After germination, the sporophyte produces haploid spores, which forms a new thallus (gametophyte).

As a result, it can be said that the summer season was the best time to perform operations (Peters et al. 2018). Similar to this observation, Lunic et al. (2022) also studied that the total phenol and flavonoid content was higher in the summer season as compared to monsoon and winter season. The increased phenolic and flavonoid content means it has significant antioxidant potential, and it can be employed in the pharmaceutical industry to develop novel medications to treat a variety of ailments.

Based on the findings, the methanolic fraction of summer season was further processed for GC-MS to get a comprehensive phytochemical profiling.

Among the identified bioactive compounds, methyl ester of hexadecanoic acid has the highest percent peak area. This compound has antifungal and antibacterial properties (Mehdi et al. 2021). Methyl stearate regulates the porosity of cell walls and protects against abiotic stress (Wu et al. 2018). Heptacosane has antibacterial and antifungal properties (Everall et al. 1996). An alkane called hexacosane has anti-inflammatory properties (Ansorena et al. 2001). Antioxidant, hypocholesterol nematicide, insecticide, and lubricant, n-hexadecanoic acid (palmitic acid) and sequisterpene caryophyllene have antifungal and antibacterial properties. Heptadecane is an alkane hydrocarbon organic molecule. Anti-inflammatory and anti-cancer activities are found in phytol (Beulah et al. 2018).

As a result, every phytochemical detected in the aqueous methanol fraction of *T. megapolitana* has its unique biological activity that is important for therapeutic purposes, demonstrating that bryophytes could be a valuable resource for the creation of new medications in the future.

For humankind, plants not only providing food and shelter, but also utilized extensively for healing purposes as herbal medicines. Hence, plants should be regarded as the most formidable chemical laboratory capable of producing an enormous number of biologically active compounds while also providing the significant benefit of serving as a renewable natural resource for these bioactive components of remarkable remedial capabilities (Commisso et al. 2021).

CONCLUSION

In the present study, the investigation demonstrated that considering the phytochemical profile, the moss *T. megapolitana* is very useful due to the presence of alkaloids, flavonoids, phenols, terpenoids, phytosterols, anthocyanins, etc. The obtained compounds have proven medicinal value and could be used in pharmaceutical preparations and can be used as antioxidant. The nine bioactive phytoconstituents were reported during this study and some of them are very potential as antimicrobial impending. However, the quantities of these phytochemicals showed the impact of the ambient environment on their production rate in plant tissue. As evident that the highest flavonoids and phenol contents were found during the summer season for both the years, indicating their correlation with water availability and elevated temperature in the ambience. In that regard this study is further indicates that the abiotic stress are correlated with the amount of defensive phytochemicals; the useful chemicals of medicinal importance hence be obtainable *in vitro* using induction of the controlled stress for their year-round supply. In this way specific phytochemicals can be produced to get the advantages of their phytochemicals after extraction. The huge and easily available diversity of bryophytes has great potential as a reservoir of precious bioactive compounds and needs further exploration in future.

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REFERENCES

Abu Bakar M.F, Abdul Karim F., Suleiman M., Isha A. & Rahmat A. 2015. Phytochemical constituents, antioxidant and antiproliferative properties of a liverwort, *Lepidozia borneensis* Stephani from Mount Kinabalu, Sabah, Malaysia. Evidence-Based Complementary & Alternative Medicine 2015. https://eurekamag.com/research/058/556/058556079.php

Akinmoladun A.C., Ibukun E.O., Afor E., Obuotor E.M. & Farombi E.O. 2007. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Scientific Research & Essays 2(5): 163–166.

Alam A. 2012. Some Indian bryophytes known for their biologically active compounds. International Journal of Applied Biology & Pharmaceutical Technology 3(2): 239–246.

Ansorena D., Gimeno O., Astiasaran I. & Bello J. 2001. Analysis of volatile compounds by GC–MS of a dry fermented sausage: Chorizo de Pamplona. Food Research International 34(1): 67–75.

Asakawa Y. & Ludwiczuk A. 2013. Bryophytes: Liverworts, Mosses, and Hornworts: Extraction and Isolation Procedures. In: Roessner U., Dias D. (Editors) Metabolomics Tools for Natural Product Discovery. Methods in Molecular Biology (Methods and Protocols), volume 1055. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-62703-577-4_1

Asakawa Y., Toyota M., Takemoto T. & Mues R. 1981. Aromatic esters and terpenoids of the liverworts in the genera *Trichocolea*, *Neotrichocolea* and *Trichocoleopsis*. Phytochemistry 20(12): 2695–2699.

Beulah G.G., Soris P.T. & Mohan V.R. 2018. GC-MS determination of bioactive compounds of *Dendrophthoe falcata* (LF) Ettingsh: An epiphytic plant. International Journal of Health Sciences & Research. 8: 261–269.

Bhadauriya G., Rathore K.S. & Singh S. 2018. Phytochemical screening and total phenolic content in the extract of bryophyte *Plagiochasma appendiculatum* and *Dicranum scoparium*. Environmental Conservation Journal 19(1-2): 175–181.

Chavhan A. 2017. Phytochemical screening and antibacterial activity of Bryophytes. International Journal of Life Sciences 5(3): 405–408.

Commisso M., Guarino F., Marchi L., Muto A., Piro A. & Degola F. 2021. Bryo-activities: a review on how bryophytes are contributing to the arsenal of natural bioactive compounds against fungi. Plants 10(2): 203.

Deora G.S. & Deora S. 2018. Studies on phytochemical screening of certain bryophytes of Rajasthan. Indian Journal of Plant Sciences 7(4): 42–48.

ENVIS-BSI. 2021. http://www.bsienvis.nic.in (data retrieved on 20.02.2021).

Everall N.C. & Lees D.R. 1996. The use of barley-straw to control general and blue-green algal growth in a Derbyshire reservoir. Water Research 30(2): 269–276.

Frahm J.P. 2004. Recent Developments of Commercial Products from Bryophytes. Bryologist 107(3): 277–283.

Glime J.M. 2017. Medical Uses: Biologically Active Substances. Chapter 2-2, Glime J.M. et al. (Editors) – Bryophyte Ecology. eBook sponsored by Michigan Technological University and the International Association of Bryologists. http://digitalcommons.mtu.edu/bryophyte-ecology/

Isah T. 2019. Stress and defense responses in plant secondary metabolites production. Biological Research 52(1): 39. https://doi.org/10.1186/s40659-019-0246-3

Karim F.A., Suleiman M., Rahmat A.S.M.A.H. & Bakar M.A. 2014. Phytochemicals, antioxidant and antiproliferative properties of five moss species from Sabah, Malaysia. International Journal of Pharmacy & Pharmaceutical Sciences 6: 292–297.

Lunic T.M., Mandic M.R., Oalðe Pavlovic M.M., Sabovljevic A.D., Sabovljevic M.S., Bozic Nedeljkovic B.D. & Bozic B.D. 2022. The Influence of Seasonality on Secondary Metabolite Profiles and Neuroprotective activities of moss *Hypnum cupressiforme* extracts: In-Vitro and In-Silico Study. Plants 11(1): 123.

Mehdi M.A.H., Al-Alawi A.H.M., Thabet A.Z.A., Alarabi F.Y., Omar G.M.N. & Pradhan V. 2021. Analysis of bioactive chemical compounds of leaves extracts from *Tamarindus indica* using FT-IR and GC-MS spectroscopy. Asian Journal of Research in Biochemistry 8(1): 22–34. https://doi.org/10.9734/ajrb/2021/v8i130171

Muhammad N., Ali N., Uddin N., Wadood S.F., Khan M.K., Alam N. & Haq I. 2018. Evaluation of informants consensus factor of medicinal uses of bryophytes in Swegalai Valley KPK, Pakistan. Journal of Biological & Environmental Sciences 12(5): 57–63

Nandy S. & Dey A. 2020. Bibenzyls and bisbybenzyls of bryophytic origin as promising source of novel therapeutics: pharmacology, synthesis and structure-activity. DARU Journal of Pharmaceutical Sciences 28: 701–734. https://doi.org/10.1007/s40199-020-00341-0

Peters K., Gorzolka K., Bruelheide H. & Neumann S. 2018. Seasonal variation of secondary metabolites in nine different bryophytes. Ecology & Evolution 8(17): 9105–9117.

Peters K., Treutler H., Döll S., Kindt A.S., Hankemeier T. & Neumann S. 2019. Chemical diversity and classification of secondary metabolites in nine bryophyte species. Metabolites 9(10): 222.

Rice S.K. 2009. Mosses (Bryophytes). In: Likens G.E. (Editor) – Encyclopedia of Inland Waters, Academic Press, pp. 88-96. https://doi.org/10.1016/B978-012370626-3.00219-2.

Sabovljevic A., Sokovic M., Glamoclija J., Ciric A., Vujicic M., Pejin B. & Sabovljevic M. 2011. Bio-activities of extracts from some axenically farmed and naturally grown bryophytes. Journal of Medicinal Plant Research 5(4): 565–571.

Sabovljevic M.S., Vujicic M., Wang X., Garraffo H.M., Bewley C.A. & Sabovljevic A. 2017. Production of the macrocyclic bis-bibenzyls in axenically farmed and wild liverwort *Marchantia polymorpha* L. subsp. *ruderalis* Bischl. et Boisselier. Plant Biosystems 151(3): 414–418.

Saini S., Alam A. & Vats S. 2022. Bioactive compounds and antioxidant capacity of selected bryophytes: relatively neglected plants. Plant Biosystems http://doi.org/10.1080/11263504.2021.2020354

Saxena D.K. 2004. Uses of bryophytes. Resonance 9(6): 56–65.

Thakur S. & Kapila S. 2017. Seasonal changes in antioxidant enzymes, polyphenol oxidase enzyme, flavonoids and phenolic content in three leafy liverworts. Lindbergia 40(5): 39–44.

Vitt D.H., Crandall-Stotler B. & Wood A. 2014. Survival in a dry world through avoidance and tolerance. pp. 267–295. In: Rajakaruna N., Boyd R. & Harris T. (Editors) – Plant ecology and evolution in harsh environments. Nova Publishers.

Vats S. & Alam A. 2013. Antioxidant activity of *Barbula javanica* Doz. et Molk.: A relatively unexplored bryophyte. Elixir Applied Botany 65: 20103–20104.

Vats S., Tiwari R., Alam A., Behera K.K. & Pareek R. 2012. Evaluation of phytochemicals, antioxidant and antimicrobial activity of in vitro culture of *Vigna unguiculata* (L.) Walp. Researcher 4: 70–74.

Wu H.C., Bulgakov V.P. & Jinn T.L. 2018. Pectin methylesterases: cell wall remodeling proteins are required for plant response to heat stress. Frontiers in Plant Science 9: 1612.

Yadav S., Srivastava A., Biswas S., Basu S., Singh S.K. & Mishra Y. 2021. Seasonal changes in the Antioxidant Defence System of a liverwort *Dumortiera hirsuta*. Journal of Plant Growth Regulation http://doi.org/10.1007/s00344-021-10379-2

Yamane Y. & Hayashi T. 2006. Evaluation of environmental conditions for the formation of severe local storms across the Indian subcontinent. Geophysical Research Letters 33: 17.

Yoshinori A., Masao T. & Tsunematsu T. 1980. Four new sacculatane-type diterpenoids from *Trichocoleopsis sacculata* and *Pellia endiviaefolia*. Phytochemistry 19(8): 1799–1803.

Zhishen J., Mengcheng T. & Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64(4): 555–559.