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Analysis of the antioxidant activity and caffeine content of *Barbula indica* (Hook.) Spreng. (Bryophyta; Pottiaceae)

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Abstract

The current research aims to investigate the phytochemicals of *Barbula indica* in different solvents, its antioxidant properties and quantification of the caffeine content in a methanolic extract by high-performance thin-layer chromatography (HPTLC). The total alkaloid content was higher in the methanolic extract, 32.06±0.28 mg/g. The antioxidant properties were evaluated by using DPPH and NOSA; the IC_{50} values of DPPH and NOSA were 61.09±1.26 µg/mL and 58.04±0.46 µg/mL, respectively. TLC and HPTLC are advanced standardization procedures that provide quantitative and semi-quantitative data about the active compound present in a sample. The development of such a biochemical fingerprint can be used to differentiate the species and adulterants through the analysis of phytochemical content and thus can serve as biochemical markers in pharmacological research and studies related to plant systematics.

Keywords

Alkaloid, antioxidant, *Barbula indica*, caffeine, HPTLC, phytochemical

Introduction

India has one of world's most diverse, ancient and distinct culture reating to the medicinal use of plants. A wide variety of medicinally important and valuable phytochemicals are used to treat a variety of illnesses. Mosses, a type of bryophytes, are terrestrial plants found all over the world that play an active role in the cycling of nutrients and water. They also play an important role in regulating weathering and yield many products (1, 2, 3). Bryophytes are employed to monitor habitats, in agricultural and urban regions and are in use as bioindicators of atmospheric conditions (4, 5).

Bryophytes do not have advanced protective features like lignified cell walls, thorns, spines, and bark which are possessed by other groups of plants. They however produce a wide range of secondary metabolites that protect plant from insects and infectious microorganisms (6, 7, 8). Benzenoids, bibenzyls and other natural products have been identified and described as bryophytes to date (9,10). Several chemicals that are biologically constituted as antifungal, antiviral, and antidiuretic agents are beneficial in the treatment of burns and bruising (11). *Rhodobryum roseum* and *R. giganteum* are commonly used in China to treat congestive heart failure; *Sphagnum* species have been used in surgical dressing pads due to their excellent permeability; and *Polytrichum commune* has been used as a homeostatic, diuretic and antipyretic substance (12).

The initial step in identifying the phytochemical constituents in a sample is the analysis using TLC and HPTLC which can provide a densitogram and a computerized chromatographic fingerprint which can be used to identify the marker component in a particular plant. Both processes can yield adequate, rapid and consistent reproducible results (13).

Methods based on HPTLC can be a potential substitute as they are being investigated as a useful technique in regular drug analysis. The capacity to evaluate multiple samples simultaneously with a modest amount of mobile phase is a major benefit of HPTLC. This can cut down the amount of time and money spent for chemical analysis. Furthermore, it can reduce the risk of exposure and significantly reduce the challenges associated with the disposal of toxic organic effluents and thus lowering the risk of environmental pollution (14).

Most bryologists in India have so far concentrated studies on morpho-taxonomy and diversification issues (15, 16, 17). As a result, currently there is a lacuna in research relating to phytochemical constituents of bryophytes in India.

With this background, qualitative, quantitative techniques including TLC, and HPTLC were used to determine the phytochemical elements such as alkaloids (caffeine) present in the extract of *B. indica*. The study is found to be crucial and important for scientifically validating folkloric claims about the value of *B. indica*.

Materials and Methods

Identification and Collection of Plant sample

B. indica (Hook.) Spreng. samples were collected from Nainital, Uttarakhand (India), during December–January 2020 (Fig.). The specimens were accurately identified based on morphological characters (18). The reference specimen's taxonomic details were submitted to the Banasthali University Herbarium, Rajasthan, India (BURI-1394/2022) at Banasthali Vidyapith.

Fig 1. *Barbula indica* population in the wild

Extraction

The plant specimens were completely cleaned with water to remove soil and other plant debris. The collected thalli were then placed in liquid nitrogen and transferred to the research lab, where it was stored at -80°C until further investigation. The thalli of *B. indica* were air-dried at room temperature and pulverized into a powder before extract preparation. Methanol, diethyl ether and hexane solvents were used separately to dissolve 5 g of powder, which was then maintained at 30 °C in an orbital-shaker for 48 hours. After filtering, the extract was kept at 4 °C until use (19).

Determination of total alkaloid content

A spectrophotometric technique was used to determine the total anti-oxidant capacity (TAC) (20, 21). This procedure is related to the response linking bromocresol green (BCG) and alkaloids. After mixing the plant sample with different solvents separately, (1 mg/mL and 2 N HCl), it was filtered. 0.1 N NaOH was used to bring the pH of the phosphate buffer combination to neutral. In a separating funnel, 5 mL of BCG solution and 5 mL of phosphate buffer were added to 1 mL of this combination. The mixture was forcefully agitated, and then chloroform was used to extract the resultant complex. The sample was taken and diluted to volume using chloroform in a 10 mL volumetric flask. The complex's absorbance in chloroform was measured at 470 nm.

Antioxidant Assay

Radical scavenging action of DPPH

For determining the effect of the crude methanolic extract on free radicals, the method described by Mukhia *et al.* (22) was used. DPPH states that in 100 mL of methanol, 4 g of DPPH was dissolved. 2 mL of DPPH solution was added to 2 mL of extract. After 30 minutes of incubation period, the decrease in the DPPH free radical was measured using a spectrophotometer (ELICO double beam, SL 210 UV Vis Spectrophotometer) at 517 nm. The scavenging activity of the methanolic extract was determined using the IC_{50} value. It's defined as a sample potency that induces a 50% reduction in oxidative radicals. The lower the IC_{50} value, the stronger the antioxidant scavenging activity. The % decolorization was used to measure the extract's scavenging activity. The sample's % decolorization was used to determine the sample's scavenging activity.

Nitric oxide scavenging assay (NOSA)

The approach of Mukhia *et al.* (22) was used to calculate the nitric oxide scavenging activity of the sample. It was incubated for 150 minutes at 25 °C in 0.5 mL of phosphate-buffered saline (1 M; pH 7.4) with 0.5 mL of the extract and 2 mL of 20 mM sodium nitroprusside. After incubation, 3 mL of Griess Reagent was added to the solution compound and allowed to stand for 30 minutes at room temperature. The combination's absorbance was measured at 540 nm. Nitric oxide radical scavenging activity was calculated and expressed as IC_{50}.

TLC (Thin Layer Chromatography) profile

TLC analysis of the sample was performed with known standards based on the results of the qualitative phyto-

chemical analysis (23). An accurately measured extract was dissolved in methanol solvent to produce a known concentration. The extract was separated into a suitable mobile phase along with the standard silica gel $60F_{254}$ aluminum sheet (3×10 cm). The sample was spotted on the aluminum sheet with a micro-capillary tube. The separation of compounds was done using different combinations of solvent systems and was tested according to their varying polarities. A chromatogram was developed with a solvent system containing a volume ratio of ethyl acetate: methanol (85:15). The TLC sheet was dipped in the solvent chamber of the selected solvent system and allowed to run up to three-fourths of the sheet. It was separated and dried with air. The sheet was kept in the UV-chamber to visualize the spots. Following that, a specific type of compound was completed, with a single compound identified as erectly segregated spots. The retention factor (R_f) for each point is comparable to the amount of space moved over the total amount of space covered by the solvent.

Profile of HPTLC (High-Performance Thin Layer Chromatography)

Preparation of Sample

In 1 mL of HPTLC-grade methanol, 5 mg of plant thalli methanolic extract was dissolved and centrifuged for 5 minutes at 3000 rpm. As an HPTLC analysis, this solution was used (24).

Developing Solvent System

The HPTLC fingerprint profile for alkaloids was developed using an ethyl acetate: methanol (85:15) solvent solution (25-27).

Sample Application

3 µL of the reference mix and 2 µL of the sample were loaded individually as 5 mm bands on loaded silica gel $60F_{254}$ aluminum sheets (3×10 cm) using a Linomat 5 applicator connected to a CAMAG HPTLC apparatus and Hamilton syringe, which was set up with winCATs software.

Chromatogram Development

The chromatogram was collected in a twin-trough glass chamber (20×10 cm) saturated with the appropriate moving phase after the spots were applied.

Detection of a Spot

The images were captured using a UV 280 nm visible range and an air-dry plate (CAMAG Reprostar 3 in an image documentation chamber). The winCATs (1.3.4 version) software was used to capture the maximum number, including its height, area, and R_f values, for fingerprint data.

Statistical Analysis

The data are shown as the average of three triplicates (n = 3). All the data were analyzed using the IBM SPSS Statistics 20 software. Three-way interactions were applied to the chosen variable. To compare the results, a one-way ANOVA was used. Tukey's P-value 0.05 Post-Hoc-Tests were used to compare the variance of data for each output variable using multiple comparison. The results are presented as a mean with a standard deviation.

Results and Discussion

The gametophytes of *B. indica* were examined in this study concerning their phytochemical content, taking the winter season into account and demonstrating their association with water availability in the environment. Alkaloid compounds were also investigated and separated from mixtures of diverse components using TLC and HPTLC.

Quantitative Analysis of alkaloid

The total alkaloid content of *B. indica* was expressed as caffeine. Alkaloid was highly present in the methanolic extract, 32.06±0.28 mg/g, rather than diethyl ether, 18.04±0.10 mg/g, and hexane, 9.02±0.06 mg/g (Fig. 2). The methanolic extract of the plant produced the best result.

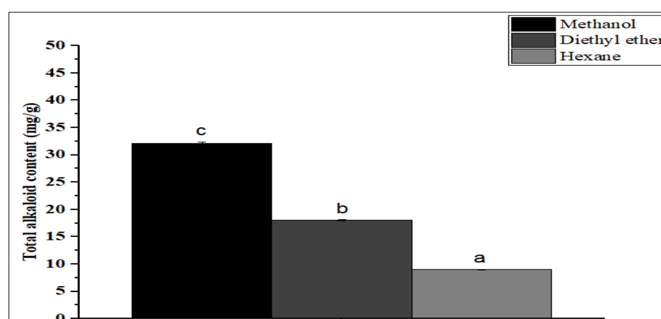

Fig 2. Quantitative analysis of the total alkaloid content of *B. indica*.

Antioxidant properties

For the DPPH assay, the IC_{50} value of *B. indica* was 61.09±1.26 µg/mL whereas, for the NOSA assay, the IC_{50} value was 58.04±0.46 µg/mL (Fig. 3).

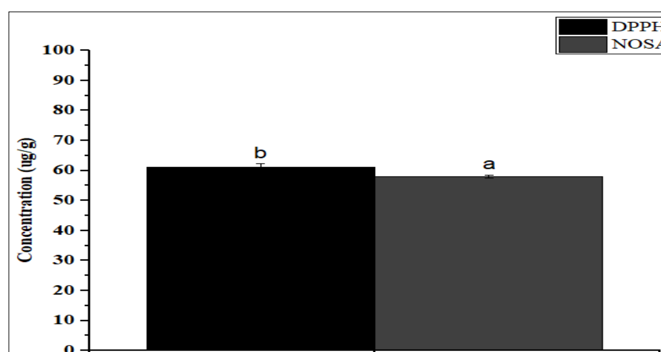

Fig 3. Antioxidant activities of *B. indica* in methanolic extract.

Thin Layer Chromatography (TLC)

The result of the separation of an alkaloid compound in a methanolic extract of *B. indica* by thin-layer chromatography shows a spot with a light violet color, and R_f values determined by the distance travelled by the solute and the distance travelled by the solvent are both measured. The standard and sample R_f values were 0.55 and 0.53, respectively, confirming the presence of an alkaloid such as caffeine (Fig. 4).

HPTLC (High-performance thin-layer chromatography)

The result from HPTLC fingerprint scanning for alkaloids such as caffeine at wavelength 280 nm in a methanolic extract of *B. indica* (Fig. 5). The R_f values in ascending order begin at 0.42 and end at 0.48, with the highest caffeine concentration being 23.95 ng, or 24.18 percent, and its corresponding R_f value being 0.47 (Table 1; Fig. 6).

Fig 4. Spot detection of *B. indica* by using TLC

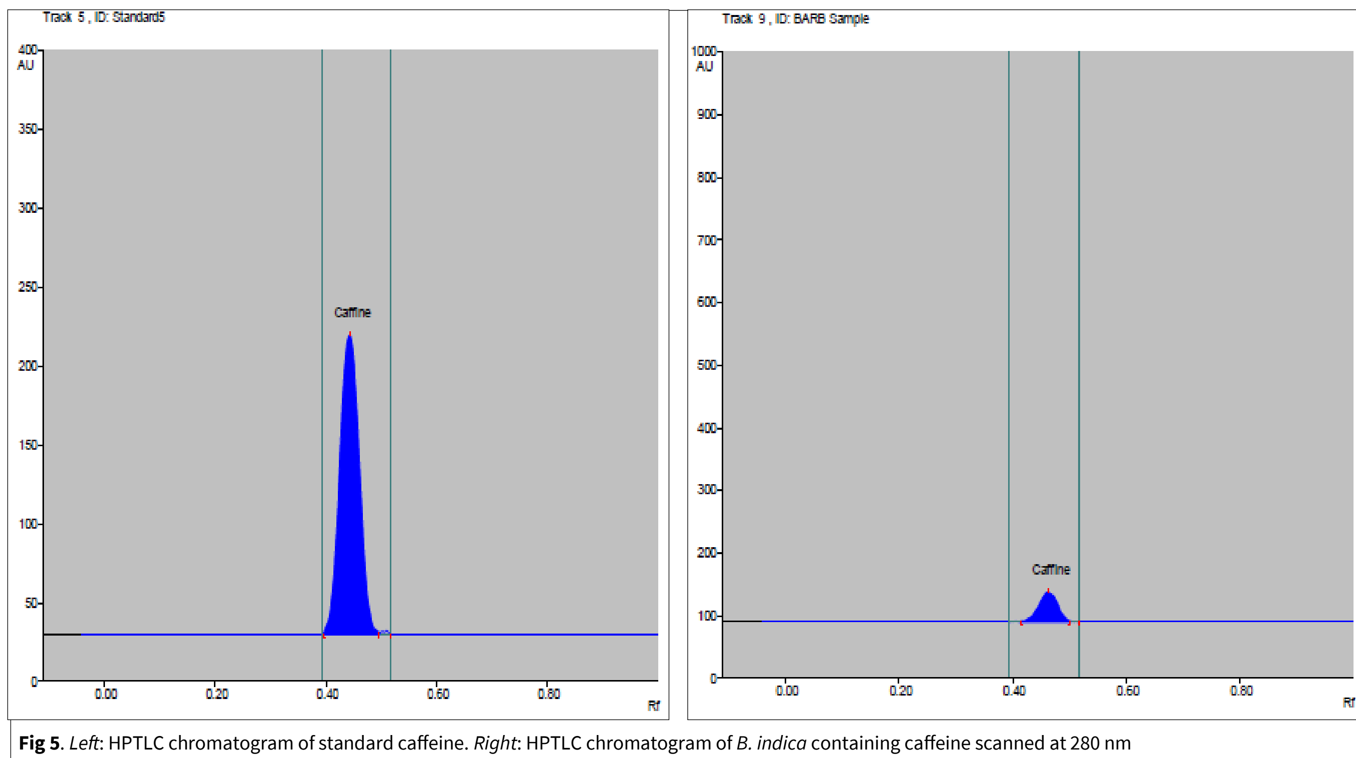

Fig 5. *Left*: HPTLC chromatogram of standard caffeine. *Right*: HPTLC chromatogram of *B. indica* containing caffeine scanned at 280 nm

Standardization and authentication measures should be used to ensure the identification, quality, purity, and safety of herbal medications. The chromatographic approach is the most generally utilized technique for general use among the several methods available for separating the plant elements (28).

This study included phytochemical profiling, antioxidant profiling, and HPTLC profiling. The antioxidant capacity of this species could be attributed to the phytochemical content, which can act as a reducing agent, as well as the synergistic effects of the alkaloid. The presence of an active metabolite in the methanolic extract of the study species, *B. indica*, was confirmed by TLC and HPTLC analysis. To separate the bioactive molecule, TLC and HPTLC used relatively high polar solvents such as ethyl acetate and methanol as mobile phases (Figs. 4–5). Many early results (29, 30) reveal that this mobile phase of high-polarity solvents can effectively separate bioactive chemicals in a variety of plant species. Alkaloids are a large and diversified collection of secondary metabolites found in almost all plants at concentrations of 10-15%. At the cellular level, it also has anti-mitotic and allergenic properties. Many alkaloids are bitter, yet they have a physiological effect that makes them useful in therapies for disorders like malaria, diabetes, cancer, and heart failure (31).

Conclusion

The present study was carried out to determine the phytochemical and antioxidant parameters of *B. indica,* in detail. The presence of diverse phytoconstituents could explain the diverse pharmacological effects of *B. indica*. Apart from other alkaloids, this study also estimated caffeine, in *B. indica*. Although this species is frequently found in the Kumaun region of Uttarakhand and though it is easy to identify and collect, few people are aware of its therapeutic benefits including that of other bryophytes. This finding can make the plant as an easily accessible choice of caffeine for the tribal people who can use them as a choice whenever caffeine is required for therapeutic purposes. In this way, the present study places this commonly growing moss species as an addition to the local herbology.

Table 1. Height and amount of caffeine present in the standard and sample.

Substance: Caffine @ 280 nm

Regression via height: Polynomial   Y = 5.499 + 2.366 * X + -0.004758 * X2   r = 0.99899 sdv = 2.44

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)	SampleID/Remark
1	1	0.46	20.00 ng	48.64				
2	1	0.43	40.00 ng	94.68				
3	1	0.44	60.00 ng	131.63				
4	1	0.44	80.00 ng	167.55				
5	1	0.44	100.00 ng	189.30				
6	1	0.45	120.00 ng	219.08				
7	1	0.45	140.00 ng	245.98				
8	2	0.46		27.62	18.00 ng			BARB Sample
9	2	0.46		46.99	18.20 ng			BARB Sample
10	2	0.48		59.43	23.95 ng			BARB Sample

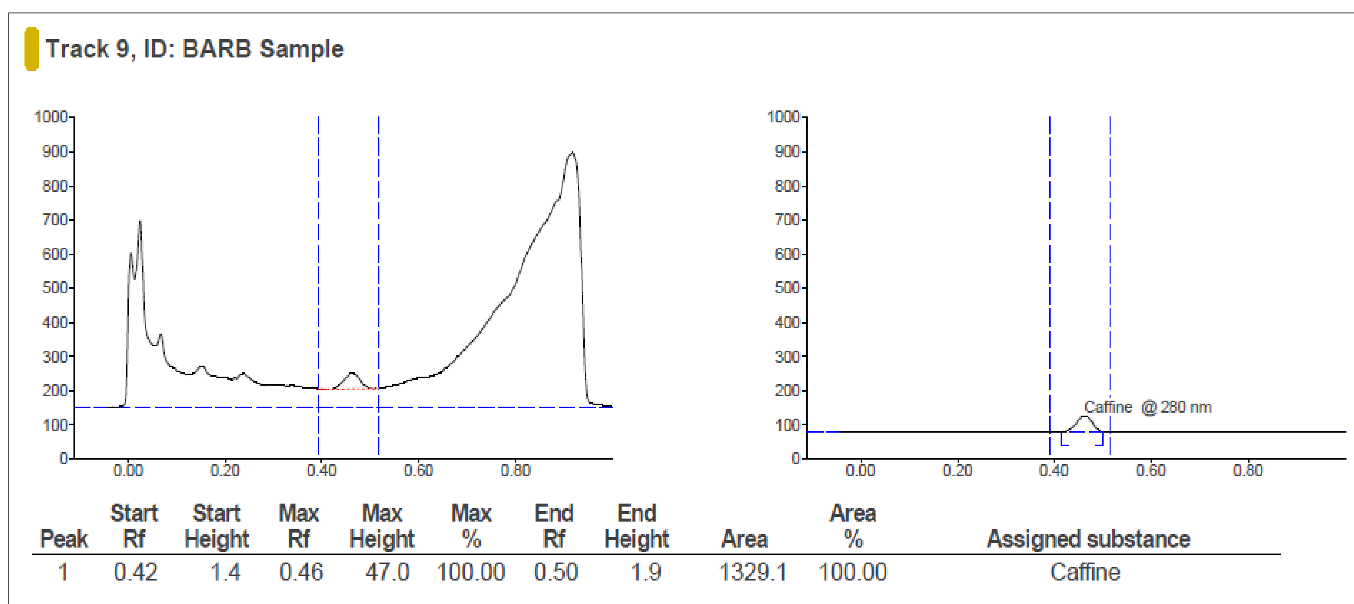

Fig 6. HPTLC chromatogram of methanolic extract of *B. indica*

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Authors contributions

All authors worked together to complete this work. The subject was created, and the plant was selected by AA. The experiments were done, the first draft was written by SJ, and the obtained results were analysed by EI. All authors reviewed and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. He X, He KS, Hyvonen J. Will bryophytes survive in a warming world? Perspect. Plant Ecol. Eyol. Syst. 2016; 19:49-60. https://doi.org/10.1016/j.ppees.2016.02.005
2. Spitale D. The interaction between elevational gradient and substratum reveals how bryophytes respond to the climate. J Veget Sci. 2016; 27:844-853. https://doi.org/10.1111/jvs12403
3. Garcia-Carmona M, Arcenegui V, Garcia-Orenes F, Mataix-Solera J. The role of mosses in soil stability, fertility and microbiology six years after a post-fire salvage logging management.

J Environ Manag. 2020; 262:110287. https://doi.org/10.1016/j.jenvman.2020.110287

4. Oishi Y, Hiura T. Bryophytes as bioindicators of the atmospheric environment in urban-forest landscapes. Landsc Urban Plan. 2017; 167:348-355. https://doi.org/10.1016/j.landurbplan.2017.07.010
5. Ah-Peng C, Cardoso AW, Flores O, West A, Wilding N, Strasberg D, Hedderson TAJ. The role of epiphytic bryophytes in interception, storage, and the regulated release of atmospheric moisture in a tropical montane cloud forest. J Hydrol. 2017; 548:665-673. https://doi.org/10.1016/j.jhydrol.2017.03.043
6. Carella P, Schornack S. Manipulation of bryophyte hosts by pathogenic and symbiotic microbes. Plant Cell Physiol. 2017; 59:656-665. https://doi.org/10.1093/pcp/pcx182
7. Asakawa Y, Ludwiczuk A. Chemical constituents of bryophytes: Structures and biological activity. J Nat Prod. 2018; 81:641-660. https://doi.org/10.1021/acs.jnatprod.6b01046
8. Ludwiczuk A, Asakawa Y. Bryophytes as a source of bioactive volatile terpenoids-A review. Food Chem Toxic. 2019; 132:110649. https://doi.org/10.1016/j.fct.2019.110649
9. Peters K, Treutler H, Doll S, Kindt SDA, Hankemeier T, Neumann S. Chemical diversity and classification of secondary metabolites in nine bryophyte species. Metabolites. 2019; 9:222. https://doi.org/10.3390/metabo9100222
10. Lu Y, Eiriksson FF, Thorsteinsdottir M, Simonsen HT. Valuable fatty acids in bryophytes—Production, biosynthesis, analysis and applications. Plants. 2019; 8:524. https://doi.org/10.3390/plants8110524
11. Sabovljevic MS, Sabovljevic AD, Ikram NKK, Peramuna A, Bae H, Simonsen HT. Bryophytes-an emerging source for herbal remedies and chemical production. Plant Genet Resour. 2016; 14:314-327. https://doi.org/10.1017/S1479262116000320
12. Chandra S, Chandra D, Barh A, Pankaj, Pandey RK, Sharma IP. Bryophytes: Hoard of remedies, an ethnomedicinal review. J Trad Compl Med. 2017; 7:94-98. https://doi.org/10.1016/j.jtcme.2016.01.007
13. Moffat CA. In: Clarke's analysis of drugs and poisons. London, Pharmaceutical Press; 2001.
14. Khandelwal KR. Practical Pharmocognosy. 11th ed. Pune:Nirali Prakashan; 2002. pp. 7-10.
15. Joshi S, Singh S, Sharma R, Vats S, Alam A. Gas chromatography-mass spectrometry (GC–MS) profiling of aqueous methanol fraction of *Plagiochasma appendiculatum* Lehm. & Lindenb. and *Sphagnum fimbriatum* Wilson for probable antiviral potential. Vegetos. 2022; (30) 1-6. https://doi.org/10.1007/s42535-022-00458-4
16. Joshi S, Singh S, Sharma R, Vats S, Nagaraju GP, Alam A. Phytochemical Screening and Antioxidant potential of *Plagiochasma appendiculatum* Lehm. & Lindenb. and Sphagnum fimbriatum Wilson. Plant Science Today. 2022; 9(4):986-90. https://doi.org/10.14719/pst.1892
17. Joshi S, Vats S, Alam A. Seasonal phytochemical screening and Gas Chromatography- Mass Spectroscopy analysis of *Timmia megapolitana* Hedw. (Timmiaceae) from Kumaun Himalayas, India. Geophytology. 2022; 51(1&2):121-128.
18. Dandotiya D, Govindapyari H, Suman S, Uniyal, PL. Checklist of the bryophytes of India. Archive for Bryology. 2011; 88(1):.1-126.
19. Vats S, Alam A. Antioxidant activity of *Barbula javanica* Doz. et Molk.: A relatively unexplored bryophyte. Elixir Appl Botany. 2013; 65:20103-20104.
20. Shamsa F, Monsef H, Ghamooshi R, Verdian-rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci. 2008; 32:17-20.
21. Sharief N, Srinivasulu A, Uma Maheshwara Rao V. Estimation of alkaloids and total phenol in roots of Derris trifoliate and evaluation for antibacterial and antioxidant activity. Indian J Appl Res. 2014; 4(5):1-3. https://doi.org/10.15373/2249555X/MAY2014/223
22. Mukhia S, Mandal P, Singh DK, Singh D, Choudhury D. In-Vitro Free-Radical Scavenging Potential of Three Liverworts of Darjeeling Himalaya. IJPSR. 2014; 5(10):4552-4561. http://dx.doi.org/10.13040/IJPSR.0975-8232.5(10).4552-61
23. Asakawa Y, Nii K, Higuchi M. Identification of sesquiterpene lactones in the Bryophyta (mosses) Takakia: Takakia species are closely related chemically to the Marchantiophyta (liverworts). Nat Prod Commun. 2015. 10 (1):1934578X1501000104. https://doi.org/10.1177/1934578X1501000104
24. Karthika K, Jamuna S, Paulsamy S. TLC and HPTLC Fingerprint Profiles of Different Bioactive Components from the Tuber of *Solena amplexicaulis*. J. Pharmacogn. Phytochem. 2014. 3 (1):198-206.
25. Wagner H, Baldt S, Zgainski EM. Plant drug analysis. 2nd Edition, Springer-Verlag, Berlin. http://dx.doi.org/10.1007/978-3-642-00574-9 Harborne JB. Phytochemical methods. 3rd ed. London: Chapman and Hall; 1998.
26. Eike Reich, Anne S. High Performance Thin Layer Chromatography for the Analysis of Medicinal Plants. New York: Thieme; 2007.p. https://doi.org/10.1055/b-0034-65188
27. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 34th ed. Pune: Nirali Prakashan; 2006. p. 377-378
28. Jeeshna MV, Paulsamy S, Mallikadevi T. Preliminary phytochemical evaluation in the leaf extracts of the plant species, *Croton bonplandianum* Baill. Plant Arch. 2010; 10:235-238.
29. Kalaiselvi M, Gomathi D, Uma C. Occurrence of bioactive compounds in *Ananas comosus* (L.): A quality standardization by HPTLC. Asian Pac J Trop Biomed. 2012; 2(3) Supplement: S1341-S1346. https://doi.org/10.1016/S2221-1691(12)60413-4
30. Nicole M, Cassiano. Alkaloids: Properties, Applications and Pharmacological Effects. New York: Nova Science Publishers, Inc; 2011. p.185. ISBN-13 9781617611308