

# Antioxidant Activities of Terpenoids from *Thuidium tamariscellum* (C. Muell.) Bosch. and Sande-Lac. a Moss

Greeshma Geetha Mohandas<sup>1</sup>, Murugan Kumaraswamy<sup>\*2</sup>

## ABSTRACT

**Introduction:** *Thuidium tamariscellum* is a small creeping epiphytic moss growing on trunks and branches of trees as well as on moist shady rocky surface at high altitudes areas. **Methods:** The present study was undertaken to analyze the phytochemicals in the species and its antioxidant potentialities in terms of DPPH, ABTS, H<sub>2</sub>O<sub>2</sub>, FRAP and metal chelating ability. **Results:** Total terpenoids level was remarkable. Interestingly, a concentration dependent free radical scavenging potential was noticed and was comparable with the synthetic antioxidant ascorbate. Further, the FTIR analysis of petroleum ether, chloroform, ethyl acetate, and methanol extract confirmed the presence of alcohols/phenols, primary, secondary amines, amides, alkanes, aldehydes, saturated aliphatic alkenes aromatics and aromatic amines, nitro compounds, carboxylic acids, esters, ethers, aliphatic amines, alkyl halides and carbonyls in the moss indicating the presence of medicinally important compounds like flavonoids, terpenoids and alkaloids in the various solvent extracts of the moss. **Conclusion:** The overall result of the present study showed that the moss is rich in important pharmaceutical compounds and was reflected as its antioxidant potential. Further studies are warranted to isolate, identify and purify the lead terpenoid present in the moss.

**Key words:** Moss, Phenols, Terpenoids, *Thuidium tamariscellum*.

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## INTRODUCTION

Bryophytes are the second largest group of plants after the flowering plants. About 20,000 members were documented from all over the world. Out of which 3000 bryophytes were reported to possess medicinal properties. They are primitive non-vascular plants and include liverworts, hornworts and mosses as three distinct groups. These plants were traditionally used in Chinese, European and Indian medicine to treat various illness including skin diseases and bronchitis. Numerous biologically active compounds were reported from these plants such as phenolic compounds, alkaloids, flavonoids and terpenoids. *Plagiochila beddomei* is a liverwort reported to possess the phenolic acids like coumaric acid, Ferulic acid, gallic acid, caffeic acid, cinnamic acid, sinapic acid and hydroxyl benzoic acid.<sup>1</sup> There is no authentic historical record, describing the application of liverworts and mosses in Ayurveda, the Indian system of medicine. Interestingly, mosses such as *Barbula*, *Fissidenc*, *Minium*, *Thuidium* and species of liverworts like *Asterella*, *Dumortiera*, *Marchantia*, *Pellia*, *Plagiochasma* and *Stephenrencella-Anthoceros* were revealed the occurrence of minerals and metals in their thallus like copper, silver, zinc, iron, lead etc.<sup>2</sup> Chinese ethnic usage revealed 40 kinds of bryophytes employed in cardiovascular diseases, tonsillitis, bronchitis, cystitis, and skin infections. Similarly, *Fissidens* and *Polytrichum* were utilized as diuretic and hair growth stimulating drugs long back of 400 years

ago. *Marchantia polymorpha* was employed in curing liver diseases like jaundice and hepatitis. In China, *Rhodobryum giganteum* and *R. roseum* were used in the treatment of heart ailments.<sup>3</sup>

*Bryum*, *Mnium*, *Philonotis* spp., and *Polytrichum juniperinum* were used by the North American, Indians to heal burns, bruises, and wounds. The Seminole people in North America were reported to use *Barbula unguiculata* as medicinal. Similarly, *Bryum capillare* and *Octoblepharum albidum* were used as febrifuge and antidote against poison. Willow moss, *Fontinalis antipyretica* was reputed as a febrifuge. Dried *Sphagnum* was used extensively as a surgical dressing material during the First World War. In France, *Marchantia polymorpha* was used to promote diuresis. In the Himalayas, *Riccia* sp. was used for the treatment of Tinea (ringworm) infestations. The use of bryophytes as antibacterial or disinfectant agents deserves special mention. *Sphagnum teres* was used in ophthalmologic diseases. In China and Bolivia, *Fissidens osmundoides* was used as an antibacterial agent to treat inflammatory conditions of the pharynx and larynx. *Haplocladium microphyllum* was used as a demulcent medicine in inflammatory conditions like bronchitis, cystitis, tonsillitis and tympanitis. *Philonotis fontana* was used by Go suite native people as a soothing preparation for healing burns.<sup>2</sup>

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Chetna Sharma *et al.*<sup>4</sup> reported that the phenolic compounds present in the methanolic extract of *Reboulia hemispherica* exhibited microbicidal potential against *Staphylococcus aureus* indicating their therapeutic potentiality. Hari Datta *et al.*<sup>5</sup> reported *Sanionia uncinata* a polar moss as an important source of natural antioxidant agents and Boris pejin *et al.*<sup>6</sup> reported the moss *Bryum moravicum* possess potential antioxidant activity. Similarly, the ethanolic extracts of *Atrichum undulatum*, *Polytrichum formosum* (Polytrichaceae) possess stronger antioxidant activity.<sup>7</sup> Fifiyana *et al.*<sup>8</sup> reported *Sphagnum cuspidatum* subsp. *subrecurvum*, *Sphagnum cuspidatum*, *Sphagnum junghuhnianum*, *Pogonatum cirratum* subsp. *fuscatum* and *Pogonatum cirratum* subsp. *macrophyllum* contained considerable amount of phenolics and flavonoids which contribute to their antioxidant properties.

Fourier Transformer Infra-red (FTIR) Spectrometry is also a valuable tool for the identification of biologically active compounds based on their functional groups and chemical compounds present in the plant extracts. But, still the experimental evidence regarding their usage among bryophytes is not fully proved. So, in this scenario, the aim of the present study was to identify the functional groups present in the various solvent extracts of *Thuidium tamariscellum* by FTIR analysis and also to evaluate the antioxidant potentiality of the moss.

## MATERIALS AND METHODS

### Plant material

*Thuidium tamariscellum* (Thuidiaceae) identity was confirmed by floras and authenticated by comparing with the herbarium of University of Calicut.

### Estimation of terpenoids

Total terpenoids was determined by the method of Ferguson.<sup>9</sup>

### Antioxidant assays

#### DPPH Radical Scavenging Assay

2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) antioxidant method as described by Blois<sup>10</sup> was used to analyze the free radical scavenging ability of the moss and the absorbance was measured at 517 nm. Ascorbic acid was used as the standard.

#### ABTS<sup>+</sup> radical scavenging assay

The 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay<sup>1</sup> is based on the oxidation of the ABTS by potassium persulfate to form a radical cation 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS<sup>+</sup>). The absorbance was taken at 734 nm and the activity was expressed as percentage inhibition of 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radicals.

#### Hydrogen peroxide radical

A solution of 40 mM H<sub>2</sub>O<sub>2</sub> was prepared in phosphate buffer (pH-7.4). 1.4 ml of different concentrations of the extracts was added to 0.6 ml of the H<sub>2</sub>O<sub>2</sub> solution. The assay mixture could stand for 10 min at 25°C and the absorbance was measured against a blank solution at 230 nm.<sup>12</sup>

#### Metal chelating activity

The chelation of ferrous ions by terpenoids extract was estimated. The absorbance of the solution was measured at 562 nm. The percentage inhibition of ferrozine-Fe<sup>2+</sup> complex formation was calculated as [(A<sub>0</sub> - A<sub>s</sub>) / A<sub>0</sub>] X 100, where A<sub>0</sub> was the absorbance of the control, and as was the absorbance of the extract/ standard.<sup>13</sup>

#### FRAP assay

The FRAP (Ferric reducing antioxidant power assay) was assayed as per the protocol of Benzie and Strain.<sup>14</sup> The absorbance of the reaction

mixture was read at 593 nm spectrophotometrically after incubation at 37°C for 10 min.

### FTIR spectroscopy

FTIR spectrometer was used to collect spectra of different solvent extracts of the moss.<sup>15</sup> The sample was mixed with KBr. The IR spectrum gives information about the functional groups. 4000 to 300 cm<sup>-1</sup> is the range of measurements. The region above 1200 cm<sup>-1</sup> shows spectral bands due to vibrations of individual functional groups whereas the region below 1200 cm<sup>-1</sup> shows bands due to vibrations of whole molecule, and is known as the fingerprint region.

## RESULTS AND DISCUSSION

Primary and secondary metabolite screening of *T. tamariscellum* revealed a tremendous range of biologically active compounds such as carbohydrates, lipid, protein, steroids, phenols, terpenoids, organic acids, sugar alcohols, fatty acids, aliphatic compounds, acetogenins, phenylquinones, and aromatic and phenolic substances which substantiates its significant bioactivities. The plant derived natural products occupy an important place in life style diseases such as cancer chemotherapy with minimal side effects.

### Total terpenoid content

Subsequently, the total terpenoid content was analyzed. Significant level of terpenoid was noticed in the moss *T. tamariscellum* i.e., 25.95 mg/g.

### DPPH scavenging effect

This is one of the most common method used to analyze the free radical scavenging ability of the plant extracts. It is a stable free radical that accepts an electron in the presence of AOX, with a subsequent discoloration of the violet colour. The discoloration was stoichiometrically related to the number of electrons accepted. A concentration dependent antioxidant activity was noticed with the terpenoid extract of the moss. The IC<sub>50</sub> value of the extract was 16 µg/ml. The following moss species such as *Sphagnum cuspidatum* subsp. *subrecurvum*, *Sphagnum cuspidatum*, *Sphagnum junghuhnianum*, *Pogonatum cirratum* subsp. *fuscatum* and *Pogonatum cirratum* subsp. *macrophyllum* displayed considerable DPPH scavenging property.<sup>8</sup> The present data was at par with that of the moss species previously reported.

### Hydrogen peroxide scavenging

The moss terpenoid extract showed a concentration dependent scavenging of the hydroxyl radical and the IC<sub>50</sub> value was 34.5 µg/ml. Hydrogen peroxide is basically a weak oxidising agent, can cross cell membranes and react with ions like Fe<sup>2+</sup> to form hydroxyl radical which is a strong oxidising agent and is toxic to the cell. This shows the moss extract can effectively scavenge hydroxyl radical. The values were statistically significant at 5% level.

### ABTS radical scavenging activity

The terpenoid extract effectively scavenged the ABTS radicals in a dose dependent manner. At 30µg/ml the inhibition was 65.45% and that of ascorbate was 79.5%. The IC<sub>50</sub> value of ascorbate was 10µg/ml while that of moss terpenoid extract was 18.5 µg/ml.

Fifiyana *et al.*<sup>8</sup> reported that the aqueous, methanolic and ethanolic extract of the moss *Pogonatum. cirratum* subsp. *fuscatum* possess strong ABTS radical scavenging activity.

### Ferric reducing antioxidant power (FRAP) assay

FRAP assay estimate the reducing potential of moss extract by reacting with a ferric tripyridyltriazine (Fe<sup>3+</sup>-TPTZ) complex to form a coloured ferrous tripyridyltriazine (Fe<sup>2+</sup>-TPTZ). Table 1 shows the ferric reducing power increased with an increase in concentration of the terpenoid

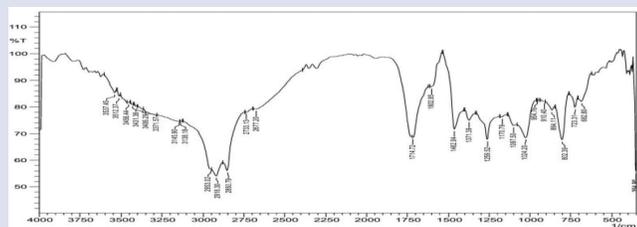
**Table 1: AOX potential of the terpenoid extract of the moss *T. tamariscellum*.**

Concentration ( $\mu\text{g/ml}$ )	Assays (% of scavenging activity)				
	DPPH scavenging effect	Hydrogen peroxide scavenging	ABTS radical scavenging activity	FRAP assay	Metal chelating activity
10	37.7 $\pm$ 4.2	30.26 $\pm$ 2.4	45.17 $\pm$ 0.89	27.10 $\pm$ 1.2	32 $\pm$ 1.46
20	56.4 $\pm$ 3.9	35.32 $\pm$ 3.8	52.07 $\pm$ 1.6	32.60 $\pm$ 1.4	34.65 $\pm$ 2.8
30	72.94 $\pm$ 6.2	47.29 $\pm$ 1.4	65.45 $\pm$ 3.4	43.3 $\pm$ 2.6	44.12 $\pm$ 4.2
40	86.16 $\pm$ 5.8	53.73 $\pm$ 6.85	74.24 $\pm$ 1.4	50.8 $\pm$ 3.2	55.28 $\pm$ 3.5
ASC (30)	88.89 $\pm$ 4.9	70.15 $\pm$ 6.4	79.5 $\pm$ 2.8	58.32 $\pm$ 5.2	58.95 $\pm$ 6.4

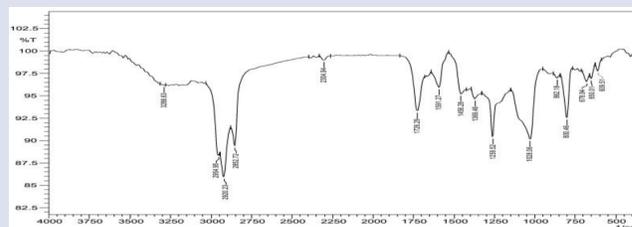
(P&lt;0.05)

**Table 2: FT-IR profile of functional groups ( $\text{cm}^{-1}$ ) in *Thuidium tamariscellum* in various solvent extracts.**

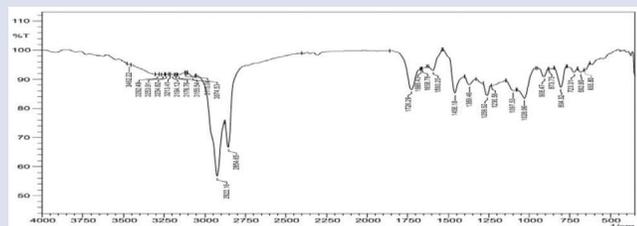
Compound	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Alcohols/phenols (O-H stretch)	3537.45,3512.37, 3456.44,3431.36 3409.29	3462.22	1028.06	3537.45,3512.3
Primary, secondary amines, amides (N-H/O-H stretch)	3456.44,3431.36 3409.29,3371.57,3145.90,3138. 18,1602.85 1462.04,864.11, 802.39	3292.49,3253.91 1593.20	3288.63,862.18 800.46,678.94	3454.51,3431.3 3404.36,3340.7 3321.42,3302.1 3286.70,3265.4 1625.99,902.69 864.11
Alkanes (C-H stretch)	2953.02,2918.30 2850.79	2922.16,2854.65 1458.18,1369.46	1456.26,1369.46, 2920.23	2931.80,2854.6
Aldehydes, saturated aliphatic alkenes (C=O stretch)	2733.13, 1714.72, 954.76	1726.29,1668.43	1726.29	1726.29
Aromatics (C-C / C-H loop) & aromatic amines (C-N stretch)	802.39,723.31 682.80	1458.18,1259.52 908.47,873.75	1591.27,1456.26 1259.52,862.18, 800.46,678.94	677.01
Nitro compounds (N=O bend)	1371.39	1369.46	1369.46	1396.46,1352.1 1342.46
Carboxylic acids (O-H stretch/ C-H stretch)	2677.20,1714.72 910.40	3234.62,3213.41 3194.12,3176.76 3155.54,3115.04 3074.53,1028.06	2954.95,2920.23 2852.72,1726.29	1041.56, 929.69
Esters, ethers (C-N stretch/ C-O stretch)	1714.72,1259.52 1170.79,1097.50 1024.20	1097.50,1028.06	1028.06	1726.29, 1273.02,1259.5
Aliphatic amines (C-N stretch)	1259.52,1170.79, 1097.50 1024.20	1230.58,1097.50 1028.06	1028.06	1041.56
Alkyl halides (C-Cl/C-Br stretch)	682.80	804.32,682.80 655.80	650.01,609.51	1273.02,1259.5 827.46,773.46, 677.01,650.01 605.65,580.57 563.21,532.35
Carbonyls (C=O stretch)	1714.72	1726.29	1726.29	1726.29
Fingerprint region	-	-	-	505.35,472.56 432.05,410.84



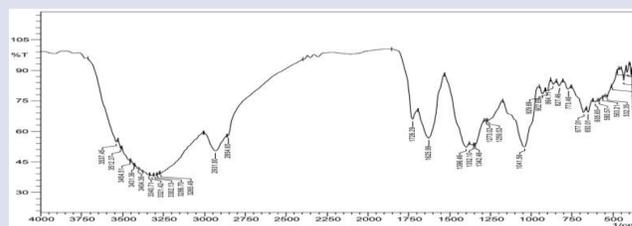
**Figure 1a:** FT-IR profile of functional groups ( $\text{cm}^{-1}$ ) in *Thuidium tamariscellum* in petroleum ether extract.



**Figure c:** FT-IR profile of functional groups ( $\text{cm}^{-1}$ ) in *Thuidium tamariscellum* in ethyl acetate extract.



**Figure b:** FT-IR profile of functional groups ( $\text{cm}^{-1}$ ) in *Thuidium tamariscellum* in chloroform extract.



**Figure d:** FT-IR profile of functional groups ( $\text{cm}^{-1}$ ) in *Thuidium tamariscellum* in methanol extract.

extract. The  $\text{IC}_{50}$  of the terpenoid extract was  $40 \mu\text{g/ml}$  while that of the synthetic antioxidant ascorbate was at  $28 \mu\text{g/ml}$ .

*Homalothecium sericeum* and *Eurhynchium striatulum* possess stronger ferric reducing power  $36.91$  and  $33.59 \mu\text{mol}/100\text{g}$ .<sup>16</sup>

#### Metal chelating activity

Table 1 shows a dose dependent increase in ferrous ( $\text{Fe}^{2+}$ ) metal ion chelation. This property is mainly due to the presence of endogenous chelating agents like terpenoids in the plant extract.  $40 \mu\text{g/ml}$  concentration of the extract showed  $55.28\%$  chelation of ferrous ions. The  $\text{IC}_{50}$  value of the moss terpenoid extract was at  $35 \mu\text{g/ml}$  while that of ascorbate was at  $22 \mu\text{g/ml}$ .

Wojtunik *et al.*<sup>17</sup> proposed a model on the antioxidant potential of terpenoids constituents in 2,2-diphenyl-1-picrylhydrazyl method. Thoppil *et al.*<sup>18</sup> reviewed the role of terpenoids as chemopreventive and therapeutic against liver cancer. Zwenger and Basu<sup>19</sup> evaluated the roles and future potentials of plant terpenoids. Partap and pandey<sup>20</sup> compared herbal antioxidants of a medicinal species. Nagarajan and Brindha<sup>21</sup> compared diterpenes therapeutic uses with special emphasis on antidiabetic activity. Topçu *et al.*<sup>22</sup> analyzed antioxidant potential of triterpenoids from *Salvia macrochlamys*. Kasote *et al.*<sup>23</sup> proved remarkable nature of polyphenolics in terms of antioxidant potential to cure many life style diseases. Polyphenolic molecules contain one or many aromatic rings with hydroxyl groups. Generally, the antioxidant capacity of the phenolics is directly related with the number of free hydroxyls and conjugation of side chains with the aromatic rings.<sup>24</sup> Flavonoids and phenolic acids of plant phenolics, are metabolically originated from the acetate via shikimate pathways, as well as the shikimate pathway from phenylalanine or tyrosine.<sup>25</sup> Phytochemicals of this group are excellent antioxidants under *in vitro* and *in vivo* experimentals. In addition, they are proven to interplay with other natural antioxidants like ascorbate or tocopherol and to synergistically enhance their biological potentials.<sup>26</sup> Flavonoids and phenylpropanoid derivatives are also oxidized by peroxidase, and act as  $\text{H}_2\text{O}_2$  scavengers.<sup>27</sup> *In vitro* experimental conditions showed that

the antioxidant power of polyphenolics is always linked to their electron donation, reducing power and metal ion chelating ability.<sup>28</sup> In this juncture, the present results were more significance in the sense that most of the proven works were from phenols or flavanoids. In the present study, the crude terpenoid content of the moss was high and displayed high antioxidant potentials.

#### FTIR analysis

The FTIR spectroscopic analysis of four different solvent extracts of *T. tamariscellum* showed the presence of various functional groups indicating the presence of bioactive compounds especially terpenes and terpenoids in the plant. Terpenoids were present with  $\text{C}=\text{O}$  stretch in  $1714.72 \text{ cm}^{-1}$  in petroleum ether extract,  $1726.29 \text{ cm}^{-1}$  in ethyl acetate, chloroform and methanol extract and with  $\text{O}-\text{H}$  stretch in  $3409.29$ ,  $3456.44$  and  $3431.36 \text{ cm}^{-1}$  in petroleum ether extract and  $3404.36$ ,  $3454.51$ ,  $3431.3 \text{ cm}^{-1}$  in methanol extract (Table 2). The presence of terpenes were also revealed due to  $\text{C}-\text{H}$  stretch in  $2953.02$ ,  $2918.30$ ,  $2850.79$  in petroleum ether extract,  $2920.23$  in ethyl acetate extract,  $2922.16$ ,  $2854.65$  in chloroform extract and  $2931.80$ ,  $2854.65$  in methanolic extract (Figure 1a,b,c,d).

Hydroxyl group ( $\sim 3400 \text{ cm}^{-1}$ ) or an oxo group (saturated  $1750-1700 \text{ cm}^{-1}$ ) indicates the presence of terpenoids.<sup>29</sup> The peak at  $2926.01 \text{ cm}^{-1}$  in the ethanolic extract of *Tylophora pauciflora* indicating the  $\text{C}-\text{H}$  stretching of alkane compounds in the rare medicinal plant.<sup>30</sup> In the methanolic extract of *Rhapis excelsa*  $\text{C}-\text{H}$  stretch at  $2856.7-2927.1 \text{ cm}^{-1}$  also reveals terpenoids in terms of  $\text{C}=\text{O}$  stretch at  $1704.18$ ,  $1708.04 \text{ cm}^{-1}$ .<sup>31</sup>

## CONCLUSION

The phytochemical study showed the presence of significant level of terpenoid in the moss which was validated by FTIR analysis showing peaks specific for terpenoids. Thus, the high antioxidant property shown by the plant is mainly due to the presence of considerable amount of terpenoids. Further studies are warranted to isolate the potential lead molecule and to evaluate its biological properties.

## ACKNOWLEDGEMENT

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## ABBREVIATIONS

**FTIR:** Fourier Transformer Infra-red; **AOX:** Antioxidant; **ABTS:** 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid assay; **DPPH:** 2,2-diphenyl-1-picryl-hydrazyl-hydrate.

## CONFLICT OF INTEREST

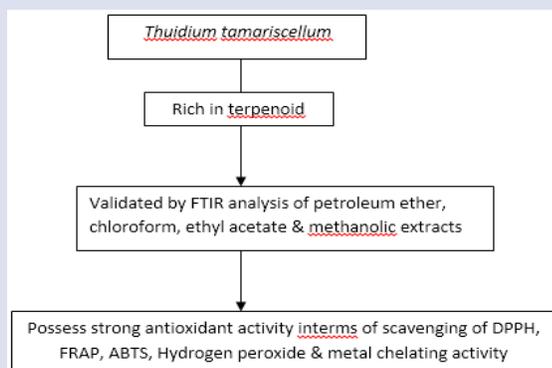
The authors declare that there are no conflict of interest pertaining to this work.

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## GRAPHICAL ABSTRACT



## SUMMARY

- The moss *Thuidium tamariscellum* is rich in secondary metabolites especially terpenoids. This data was validated by FTIR analysis showing the presence of various functional groups indicating the presence of terpenes and terpenoids. Further, the effective scavenging of DPPH, ABTS, FRAP, Hydrogen peroxide and metal chelating activity by the terpenoid extract from the moss clearly establishes the antioxidant power of the bryophyte.

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