



RESEARCH ARTICLE

Comparative study of phytochemical properties and antioxidant potential of *Plagiochasma appendiculatum* Lehm. & Lindenb. and *Riccia aravalliensis* Pande & Udar

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Received: 18 September 2025; Accepted: 12 January 2026; Available online: Version 1.0: 18 March 2026

Cite this article: Tripti S, Supriya KS, Afroz A. Comparative study of phytochemical properties and antioxidant potential of *Plagiochasma appendiculatum* Lehm. & Lindenb. and *Riccia aravalliensis* Pande & Udar. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.11842>

Abstract

Plants have long served as an important source of medicinal agents and bioactive compounds. This has led to using numerous medicinal plants with therapeutic properties due to their valuable bioactive compounds. Bryophytes, a group of non-vascular plants, have been reported to exhibit diverse therapeutic activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer and antidiabetic effects. *Plagiochasma appendiculatum* Lehm. & Lindenb. and *Riccia aravalliensis* Pande & Udar, 2 tropical bryophyte species, were examined for their phytochemical composition and antioxidant activity. Qualitative phytochemical screening revealed the presence of various bioactive compounds in both species, with some variations across different solvent extracts. Quantitative analysis showed that *P. appendiculatum* generally had higher total phenolic content (TPC) and total flavonoid content (TFC) compared to *R. aravalliensis*. Ethanol extracts demonstrated the highest TPC and TFC for both species. Antioxidant assays using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide scavenging methods indicated that *P. appendiculatum* possessed stronger antioxidant potential than *R. aravalliensis*, with ethanol extracts exhibiting the highest antioxidant activity, followed by methanol and chloroform extracts. These findings suggest that both bryophyte species, particularly *P. appendiculatum*, are rich sources of phenolic and flavonoid compounds with significant antioxidant potential. The results highlight the potential of these non-vascular plants as valuable sources of natural antioxidants and bioactive compounds, warranting further research to isolate and characterise specific bioactive molecules and elucidate their therapeutic potential in managing oxidative stress-related conditions. This study contributes to the growing evidence supporting the medicinal value of bryophytes and their potential applications in ethnomedicine and drug discovery.

Keywords: antioxidant; bioactivity; bryophytes; ethnomedicine; secondary metabolites

Introduction

Antioxidants derived from plants play a crucial role in protecting cells from oxidative stress, a major contributor to aging and the development of diseases such as cancer and cardiovascular disorders. Consequently, the identification of natural antioxidant sources has become a key focus of contemporary research. Although higher plants have traditionally been explored for their antioxidant potential, the need to discover novel and efficient natural sources remains pressing.

Bryophytes, non-vascular cryptogams comprising mosses, liverworts and hornworts represent a promising yet underexplored group in this context. Owing to their simple morphology and exposure to harsh environmental conditions, bryophytes synthesise a wide array of secondary metabolites that provide protection against ultraviolet radiation, temperature extremes, desiccation, microbial attack and herbivory. These secondary metabolites, which are derived from primary metabolic pathways but do not directly govern growth or reproduction, are well known for their diverse bioactivities, including antioxidant, antimicrobial, antifungal and anticancer properties (1, 2). Notably, several studies have demonstrated that the antioxidant potential of bryophytes can

exceed that of many higher plants (3, 4). The qualitative and quantitative composition of bryophyte secondary metabolites varies considerably among species and is influenced by ecological factors such as geographical distribution, seasonality, moisture availability and nutrient status. This biochemical diversity enhances their potential as valuable natural antioxidant reservoirs (5). Major classes of secondary compounds reported in bryophytes include aromatic compounds, terpenoids and fatty acids, with aromatic compounds and terpenoids being particularly prominent (6, 7).

Despite growing global interest in the pharmacological properties of bryophytes, studies in India have largely focused on bryofloristic surveys, with limited attention given to their biochemical and antioxidant characteristics. To bridge this knowledge gap, the present study investigates the antioxidant potential of two thalloid liverworts, *Plagiochasma appendiculatum* Lehm. and *Riccia aravalliensis* Pande and Udar. Methanolic and ethanolic extracts of these relatively underexplored species were prepared and subjected to phytochemical screening. Their antioxidant activity was evaluated *in vitro* using free-radical scavenging assays, specifically the DPPH and nitric oxide scavenging activity (NOSA) assays. This study aims to contribute to a better understanding of the antioxidant potential and biological

significance of these liverwort species.

Materials and Methods

Plant collection and authentication

In December 2024, during the winter season, samples of the selected bryophytes were collected from the Mount Abu region of Rajasthan, India, at an approximate altitude of 1722 m above sea level (Fig. 1). The collection took place between 11:00 am and 1:30 pm (IST), ensuring that all samples were obtained under optimal environmental conditions. A random sampling method was employed over a stretch of 1 km.

The identification of the samples was conducted using available herbarium specimens and literature at the Biotechnology Laboratory, Banasthali Vidyapith, Rajasthan, India. The taxonomic data of the reference specimens [BURI-4051/2024 (*P. appendiculatum*) and BURI-4052/2024 (*R. aravalliensis*)] (Fig. 2) were subsequently submitted to the Herbarium at Banasthali University, Rajasthan, India (BURI).

Preparation of plant material

Initially, soil and other plant debris were meticulously removed from the plants using clean water. The collected thalli were subsequently placed in liquid nitrogen and transported to the research laboratory, where they were preserved at a

temperature of $-80\text{ }^{\circ}\text{C}$ until further analysis. Prior to extract preparation, the thalli samples were air-dried at ambient temperature and then pulverised. The resulting powder (5 g) was macerated in methanol and left for 48 hr in an orbital shaker at $50\text{ }^{\circ}\text{C}$. Following filtration, the extract was stored at $4\text{ }^{\circ}\text{C}$ until required (8).

Qualitative phytochemical analysis

Phytochemical analysis was carried out by following the standard protocol to detect the presence of alkaloids, cardiac glycosides, carbohydrates, tannins, steroids, flavonoids, phenols, saponins, fixed oils and fats (9).

Quantitative analysis

Preparation of standard solution

Gallic acid and quercetin, both measured approximately 10 mg, were precisely weighed into dry, clean volumetric flasks. Then, methanol was used to dissolve them and the volume was increased to 10 mL using the same solvent to achieve a solution concentration of 1 mg/mL.

Preparation of test sample

10 mg of dehydrated hydro-methanolic extract was diluted in 10 mL of methanol to produce a stock solution containing 1 mg/mL.

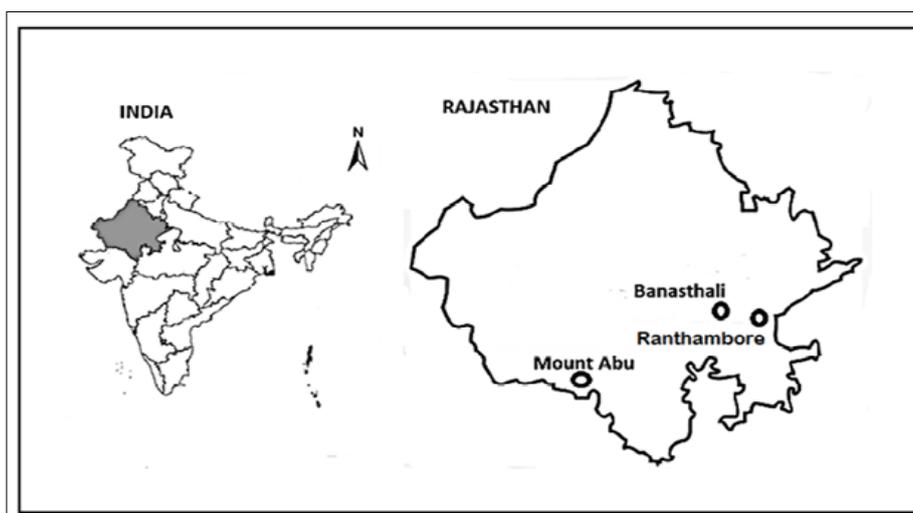


Fig. 1. Location of three areas in Rajasthan from where plant specimens were collected. 1. Banasthali Vidyapith campus at an altitude of 320 m ($25^{\circ}41'$ to $26^{\circ}24'$ N, $75^{\circ}19'$ to $76^{\circ}16'$ E); 2. Mount Abu, western Rajasthan at an altitude of ca. 1600 m ($24^{\circ}31'$ to $24^{\circ}43'$ N, $72^{\circ}38'$ to $72^{\circ}53'$ E); and 3. Ranthambore forest, at an altitude of ca. 400–500 m, 26.0173° N and 76.5026° E.

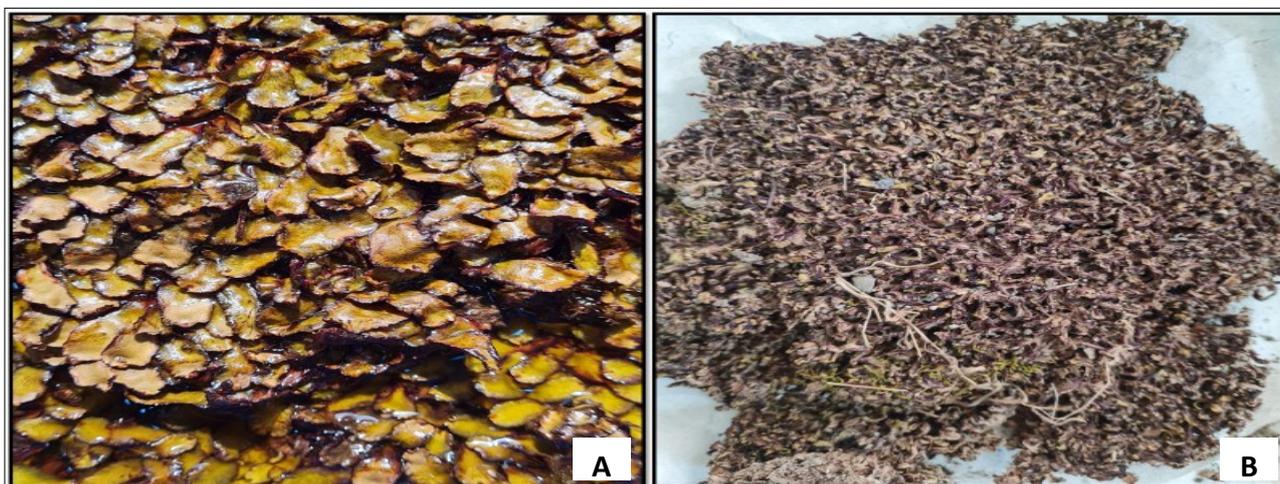


Fig. 2. (A) *Plagiochasma appendiculatum*; (B) *Riccia aravalliensis*.

Determination of total flavonoid content (TFC)

The estimation of TFC in methanol extracts from all samples was conducted using a slightly modified version (10). A 500 µg/mL extract solution was made by dissolving each plant extract in methanol. Then, 3.4 mL of aqueous methanol (30 % v/v) was added to 300 µL of extract that was taken in a test tube. Subsequently, each test tube was filled with 150 µL of a 0.5 M aqueous NaNO₂ solution and 150 µL of a 0.3 M aluminium chloride solution. About 1 mL of a 1 M NaOH solution was added after 5 min and then the mixture was thoroughly mixed before the absorbance at 506 nm was measured using a UV-visible spectrophotometer in comparison to a blank. The same process was used to set up the blank, but an equivalent volume of methanol was used instead of the plant extract. By using the same process, a Quercetin standard curve was also produced to calculate the concentration of the total flavonoid, which was then represented as mg of quercetin equivalent (QE) per gram of extracts. Triplicates of the total flavonoids in the extracts and standards were measured (11).

Determination of total phenol content (TPC)

The most popular technique for quantifying phenolic content is spectrophotometric analysis. Folin-Ciocalteu colorimetric method was used to measure the total phenol content of the selected plant leaf extract spectrophotometrically. Gallic acid was used as the standard and the results were expressed as gallic acid equivalent (GAE) per gram of sample. Methanol was used to prepare gallic acid in different concentrations (0.01-0.1 mg/mL). Then 2 mL of Folin-Ciocalteu reagent (1:10 in deionised water) and 4 mL of a saturated solution of Na₂CO₃ (7.5 % w/v) were combined with aliquots of 0.5 mL each of the test sample and each sample of the standard solution. Silver foil was placed over the tubes and they were allowed to sit at room temperature for half an hour while being periodically shaken. Methanol as a blank was used to measure the absorbance at 650 nm. Each sample underwent 3 replications of analysis (12).

Antioxidant assay

Samples for the DPPH and NOSA tests were suitably diluted using 50 % (v/v) methanol. Rutin and ascorbic acid were utilised as standard antioxidants.

DPPH radical scavenging assay

The DPPH test method is based on the reduction of a stable free radical, DPPH. It is a stable free radical that interacts with antioxidants when a hydrogen donor is present, then it becomes paired off and is reduced to the DPPH, which causes the absorbance from the DPPH to drop. A greater degree of discoloration indicates stronger free radical scavenging activity of the extract. Reduction of DPPH radicals results in a decrease in absorbance due to the conversion of the violet-coloured radical to a pale yellow non-radical form. The standard approach was followed in evaluating the plant extract's ability to scavenge DPPH radicals. Approximately 2 mL of any plant extract solution was added to 2 mL of 0.1 mM DPPH solution in methanol in a typical experiment. The mixture was then incubated for 30 min at 37 °C in a dark environment. Methanol was used as a blank to measure the absorbance at 517 nm. The DPPH radical scavenging activity percentage was determined as follows:

Percentage (%) of DPPH radical scavenging assay = $[(A_0 - A_1)/A_0] \times 100$

Where A₀ was the absorbance of the control and A₁ was the absorbance of the treated sample (13).

Nitric oxide radical scavenging assay (NOSA)

The process relies on the idea that, in an aqueous solution at physiological pH, sodium nitroprusside (SNP) spontaneously produces NO. Griess reagent can be used to calculate the nitrite ions that are produced when this oxide combines with oxygen. Then, 50 µL of each concentration of the plant extracts that had been previously dissolved in dimethyl sulphoxide (DMSO) and ascorbic acid (the reference component) were added to separate tubes. The volume was then evenly increased to 150 µL with methanol. A 2.0 mL solution of 10 mM SNP in PBS (phosphate buffer saline) was added to every tube. The solutions were incubated for 150 min at room temperature. A similar procedure was carried out again using methanol as the control blank. Each tube, including the control, got 5 mL of Griess reagent (1 % sulfanilamide in 5 % H₃PO₄ and 0.1 % NEDD (naphthylethylene diamine dihydrochloride) after the incubation period. Using a UV-visible spectrometer, the absorbance of the purple azo dye chromophore, which was created when nitrite ions were diazotised with sulphanilamide and then coupled with NEDD, was measured at 546 nm. To calculate the quantity of nitrite generated in the presence or absence of the plant extract, a standard curve based on NaNO₂ solutions with known values was used. Every experiment was conducted a minimum of 3 times and the results were displayed as the mean of 3 separate assessments (14). The IC₅₀ value for each test compound and the standard preparation were determined as follows:

Percentage scavenging = $[\text{Absorbance of control} - \text{Absorbance of test sample} / \text{Absorbance of control}] \times 100$

Statistical analysis

All experiments were performed in triplicate (n = 3). Results are presented as mean ± SD. Statistical comparisons among solvents and species were conducted using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$. Analyses were performed using GraphPad Prism/SPSS software.

Results

Qualitative analysis

Phytochemical active compounds of *P. appendiculatum* and *R. aravalliensis* were qualitatively analysed from leaves of different extracts. Results showed that *P. appendiculatum* contains carbohydrates, alkaloids, phenolic compounds, flavonoids, saponins, tannins and phytosterol (Table 1). While *R. aravalliensis* possesses alkaloids, carbohydrates, phenolic compounds, glycosides, phytosterol, flavonoids, tannins and fixed oil and fats.

Quantitative analysis

Determination of total phenolic content (TPC)

Assessing the phenolic content of the samples is done by TPC activity. Plant-based phenolic compounds possess redox characteristics that enable them to function as antioxidants. In comparison with the methanol and chloroform extracts, the ethanol extract demonstrated a higher TPC as shown in Fig. 3, measuring roughly 98.6 ± 0.077 mg GAE/g (ethanol), 88 ± 0.0056 mg GAE/g (methanol) and 73 ± 0.017 mg GAE/g (chloroform) (Table 2) for *P. appendiculatum*. In *R. aravalliensis*, the

Table 1. Phytochemical constituents in different extracts of leaf of *Plagiochasma appendiculatum* and *Riccia aravalliensis*

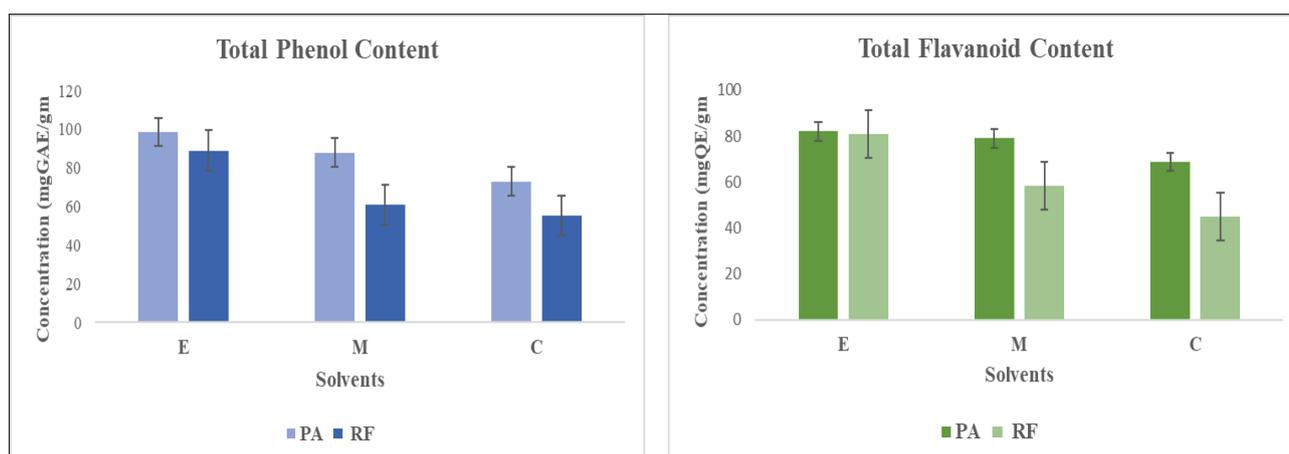
Phytochemical constituents	Test	Ethanol extract		Methanol extract		Chloroform extract	
		PA	RF	PA	RF	PA	RF
Carbohydrate	Fehling's test	++	-	+	-	+	-
	Iodine test	+	-	++	+	+	-
	Molisch's test	+	++	+	+	++	+
Alkaloids	Mayer's test	+++	+	++	+	+	-
	Hager's test	++	+	++	+	++	-
Glycosides	Borntrager's test	+	-	+	-	-	-
	Killer-Killani test	-	+	-	-	-	-
Phenolic compounds	Lead acetate test	++	++	++	+	++	+
	FeCl ₃ test	++	+	+	+	+	-
Saponins	Foam test	++	-	+	-	++	-
	Alkaline reagent test	+	+	++	+	+	+
Flavanoids	Lead acetate test	+++	+	+++	+	++	-
	Zinc test	-	++	++	-	++	+
Fixed oils and fats	Spot test	-	-	-	+	-	-
	Saponification test	-	-	+	-	-	-
Tannins	Gelatin test	++	+	++	+	++	+
Phytosterols	Salkowski's test	+	+	-	+	++	-

+++ = highly present, ++ = moderately present, + = present, - = absent. PA: *Plagiochasma appendiculatum*; RF: *Riccia aravalliensis*.

Table 2. Total phenol and flavonoid content of *Plagiochasma appendiculatum* and *Riccia aravalliensis* extract in different solvent

Variables	Leaf extracts of PA mg/g			Leaf extracts of RF mg/g		
	E	M	C	E	M	C
TPC	98.6±0.077 ^c	88±0.0056 ^b	73±0.017 ^a	89.02±0.006 ^c	61±0.015 ^b	55.3±0.001 ^a
TFC	82.02±0.0019 ^c	79±0.0036 ^b	68.8±0.0056 ^c	80.8±0.001 ^c	58.2±0.002 ^b	44.95±0.02 ^a

Values are presented as mean±SD. SD= standard deviation, TPC=total phenol content, TFC=total flavonoid content E=ethanol, M=methanol and C=chloroform.

**Fig. 3.** Comparison of total phenolic and total flavonoid contents of *Plagiochasma appendiculatum* and *Riccia aravalliensis* leaf extracts in different solvents.

ethanolic extract showed the highest total phenolic content (89.02 ± 0.006 mg GAE/g), followed by the methanolic extract (61 ± 0.015 mg GAE/g) and chloroform extract (55.3 ± 0.001 mg GAE/g), as presented in Table 2. When comparing the two plants, *P. appendiculatum* showed high TPC. The higher phenolic content observed in *P. appendiculatum* is likely a key contributor to its enhanced bioactivity, particularly its antioxidant potential. Therefore, it is anticipated that the antioxidant and antibacterial properties of this extract would perform well. These phenolic chemicals are safe for human health.

Determination of total flavonoid content (TFC)

Similar trends were also observed between the TFC and TPC values. The highest TFC value was obtained for ethanolic extract, followed by methanolic extract while the chloroform extract showed the lowest flavonoid content for both *P. appendiculatum* and *R. aravalliensis*. The TFC values were as follows 82.02 ± 0.0019 mg QE/g for ethanolic extract, 79 ± 0.0036 mg QE/g for methanolic extract and 68.8 ± 0.0056 mg QE/g for chloroform extract (Table 2). The same trend also follows in *R. aravalliensis* as in TPC. In comparison to both methanol and chloroform, the TFC

for the ethanolic extract was greater at 80.8 ± 0.001 mg QE/g, 58.2 ± 0.002 mg QE/g for the methanolic extract and 44.95 ± 0.02 mg QE/g for the chloroform extract. Similarly, *P. appendiculatum* also exhibited higher TFC as compared to *R. aravalliensis*.

Antioxidant assay

DPPH radical scavenging assay

Antioxidants found in plants naturally help to lessen or even completely eradicate the harmful effects of oxidative stress. Among many other tests, the DPPH assay is a handy tool for figuring out how antioxidant-potent plants are. By using the DPPH test, the antioxidant activity of *P. appendiculatum* and *R. aravalliensis* was assessed as shown in (Table 3). Their reducing power was calculated based on the concentration at which they provided 50% inhibition (IC_{50}) values, or, to put it another way, the quantity needed to scavenge 50% of the DPPH free radicals. Lower radical scavenging activity or reduced antioxidant capability is indicated by a higher IC_{50} value. When compared to methanol and chloroform (Fig. 4), the ethanolic extracts from

Table 3. IC₅₀ value of leaf extracts of *Plagiochasma appendiculatum* and *Riccia aravalliensis* in different solvent

Variables	Leaf extracts of PA µg/mL			Leaf extracts of RF µg/mL		
	E	M	C	E	M	C
DPPH	50.01±0.02 ^a	54.84±.016 ^b	65.69±0.023 ^c	53.97±0.017 ^a	57.04±0.025 ^b	75.78±0.005 ^c
NOSA	50.7±0.024 ^a	56.95±0.017 ^b	63.85±0.023 ^c	52.9±0.011 ^a	59.78±0.010 ^b	79.4±0.005 ^c

DPPH: 2,2-diphenylpicrylhydrazine assay; NOSA: Nitric oxide radical scavenging assay; E: ethanol; M: methanol; C: chloroform.

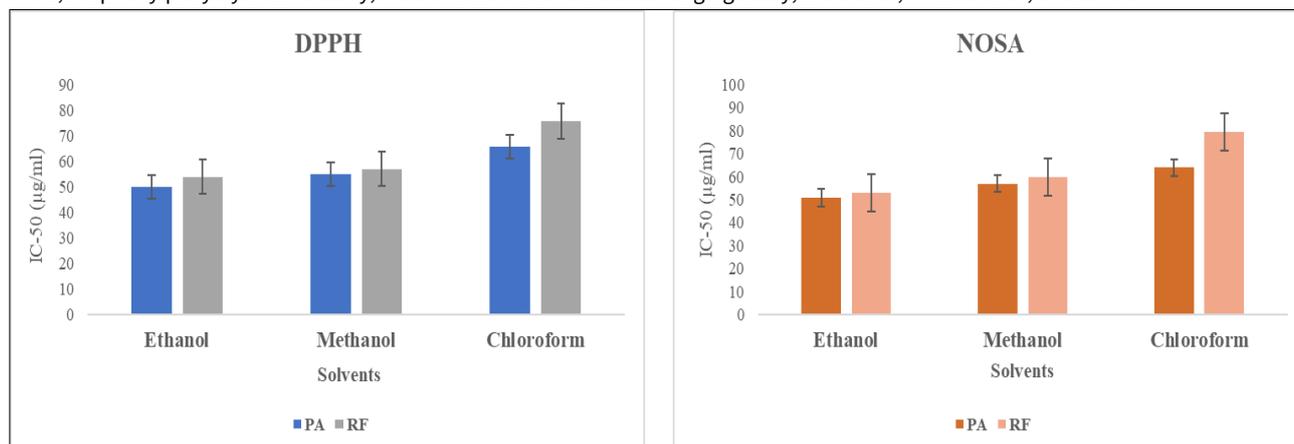


Fig. 4. Comparison of antioxidant activity of *Plagiochasma appendiculatum* and *Riccia aravalliensis* in different solvents by DPPH assay and NOSA assay.

DPPH: 2,2-diphenylpicrylhydrazine assay; NOSA: Nitric oxide radical scavenging assay; PA: *Plagiochasma appendiculatum*; RA: *Riccia aravalliensis*

P. appendiculatum and *R. aravalliensis* exhibited the best antioxidant capacity. The IC₅₀ value of the methanolic extract was 50.01 µg/mL and 53.97 µg/mL respectively, for *P. appendiculatum* and *R. aravalliensis* leaf extract. The methanol extract shows moderate antioxidant activity with the IC₅₀ value of 54.84 µg/mL and 57.04 µg/mL respectively, for *P. appendiculatum* and *R. aravalliensis* extracts. The chloroform extract shows the lowest antioxidant activity with the IC₅₀ value of 65.69 µg/mL and 75.78 µg/mL respectively, for *P. appendiculatum* and *R. aravalliensis* extracts. As a result, *P. appendiculatum* shows higher antioxidant potential than *R. aravalliensis*.

Nitric oxide scavenging assay

In aerobic environments, nitric oxide is an extremely unstable molecule. Through the intermediates N₃O₄, N₂O₄ and NO₂, it interacts with O₂ to form the stable products NO₃ and NO₂. Griess reagent (GR) was used to estimate it. The quantity of nitrous acid lowers when a test compound a scavenger is present. The degree of scavenging is reflected in the magnitude of the drop. The percentage inhibition of ethanol, methanol and chloroform extracts of *P. appendiculatum* of 3 parallel readings (Table 3) showed IC₅₀ values of 50.7, 56.95 and 63.85 µg/mL respectively. The methanolic extract of this plant shows greater antioxidant activity. The percentage inhibition of ethanol, methanol and chloroform extracts of *R. aravalliensis* of 3 parallel readings showed IC₅₀ values of 52.9, 59.78 and 79.4 µg/mL respectively. The greater antioxidant activity in this plant was shown by methanolic extract in Fig. 3.

Discussion

The present study provides a comparative evaluation of the phytochemical composition and antioxidant potential of two thalloid liverworts, *P. appendiculatum* and *R. aravalliensis*, using qualitative screening, total phenolic and flavonoid quantification and free radical scavenging assays. Bryophytes remain an underexplored group in phytochemical and antioxidant research compared to vascular plants and therefore, the findings contribute

meaningful baseline data toward understanding their bioactive potential.

Phytochemical profile and its relevance to bioactivity

Qualitative phytochemical analysis revealed that *P. appendiculatum* possessed a broader and comparatively richer spectrum of secondary metabolites, including alkaloids, phenolics, flavonoids, saponins, tannins and phytosterols, across all solvent extracts. In contrast, *R. aravalliensis* showed the presence of several similar classes but lacked saponins and exhibited weaker reactions for alkaloids and phenolics in chloroform extracts. Previous studies on bryophytes such as *Marchantia polymorpha* L., *Plagiochasma rupestre* (J.R. Forst. & G. Forst.) Steph. and *Riccia gangetica* Ahmad ex L. Söderstr., A. Hagborg & von Konrat have reported that the abundance of phenolic compounds, flavonoids and terpenoids strongly correlates with antioxidant and antimicrobial activities (15, 16). The stronger qualitative presence of phenolics and flavonoids in *P. appendiculatum* therefore provides a biochemical basis for its superior antioxidant performance observed in the present study.

Comparison of total phenolic content (TPC) and total flavonoid content (TFC) with other bryophytes and vascular plants

Quantitative analysis demonstrated that *P. appendiculatum* exhibited consistently higher TPC and TFC than *R. aravalliensis* across all solvents. The ethanolic extract of *P. appendiculatum* showed the highest TPC (98.6 mg GAE/g) and TFC (82.02 mg QE/g), which is comparable to or higher than values reported for other bryophytes such as *Marchantia polymorpha* L. (45–78 mg GAE/g) and *Polytrichum juniperinum* Hedw. (60–85 mg GAE/g) (16, 17).

When compared with certain vascular medicinal plants, the phenolic and flavonoid levels of *P. appendiculatum* fall within a biologically significant range, although generally lower than highly phenolic-rich angiosperms such as *Camellia sinensis* (L.) Kuntze or *Ocimum sanctum* L. (18). This comparison highlights bryophytes as moderate but ecologically resilient sources of

antioxidants, supporting the hypothesis that non-vascular plants compensate for the absence of structural defenses by producing potent secondary metabolites.

Antioxidant activity

The antioxidant assays (DPPH and NOSA) showed lower IC₅₀ values for *P. appendiculatum* compared to *R. aravalliensis*, indicating stronger free radical scavenging capacity. The ethanolic and methanolic extracts of *P. appendiculatum* exhibited IC₅₀ values (~50 µg/mL) comparable to those reported for *Plagiochasma rupestre* and *M. palmata* in earlier studies (18, 19). In contrast, *R. aravalliensis* showed relatively higher IC₅₀ values, suggesting weaker antioxidant efficacy.

The observed solvent-dependent variation, with ethanol and methanol extracts performing better than chloroform, is consistent with previous bryophyte studies and can be attributed to the higher solubility of polar phenolics and flavonoids in polar solvents (17).

Higher potency of *Plagiochasma appendiculatum*

The superior antioxidant potential of *P. appendiculatum* compared to *R. aravalliensis* may be explained by several factors. Ecologically, *P. appendiculatum* often inhabits exposed rocky substrates where it is subjected to intense UV radiation, desiccation stress and temperature fluctuations. Such harsh microhabitats are known to induce enhanced synthesis of phenolic compounds as protective antioxidants. Phylogenetically, species within the genus *Plagiochasma* are reported to produce unique terpenoids and phenolic derivatives not commonly found in *Riccia* species, which may contribute to stronger bioactivity (20). These adaptive and evolutionary differences likely underlie the observed variation in antioxidant performance between the 2 taxa.

Novelty and practical implications

The present study is among the few reports providing comparative antioxidant profiling of *P. appendiculatum* and *R. aravalliensis* from the Aravalli region. The demonstrated antioxidant potential, particularly of *P. appendiculatum*, suggests its possible application as a natural source of antioxidants in pharmaceutical, nutraceutical, or cosmetic formulations. Moreover, the study reinforces the importance of bryophytes as reservoirs of bioactive compounds and encourages further investigations involving compound isolation, *in vivo* bioassays and toxicity studies. The findings add novel data to bryophyte phytochemistry and support their inclusion in future bioprospecting programme.

Conclusion

This study was undertaken to conduct a comparative analysis of the phytochemical composition and antioxidant activity of 2 tropical bryophyte species, *P. appendiculatum* and *R. aravalliensis*. Both species contained diverse bioactive compounds, though their distribution varied across solvent extracts. *P. appendiculatum* showed higher levels of total phenolics and flavonoids, especially in ethanol extracts, which also displayed the strongest antioxidant activity. Antioxidant assays confirmed that *P. appendiculatum* had greater scavenging potential than *R. aravalliensis*. These results highlight the promise of bryophytes, particularly *P. appendiculatum*, as natural sources of antioxidants. Overall, this study strengthens

the growing body of evidence supporting the medicinal relevance of bryophytes and underscores their potential applications in ethnomedicine and drug discovery. The study is limited to *in vitro* antioxidant assays and lacks compound-level characterisation and *in vivo* validation. Future studies should focus on isolation of active constituents, mechanistic antioxidant pathways and pharmacological evaluation to substantiate the therapeutic potential of these liverworts.

Acknowledgements

The authors would like to express their gratitude to Prof. Ina Aditya Shastri, Vice-Chancellor, Banasthali Vidyapith, Rajasthan, for her support and encouragement. We also thank DST for giving networking support through the FIST programme at the Department of Bioscience and Biotechnology, Banasthali, along with the Bioinformatics Centre, Banasthali Vidyapith, funded by DBT.

Authors' contributions

TS and SKS carried out the plant sampling, study of the methodologies, drafted the manuscript and performed the statistical analysis. AA conceived of the study and participated in its design and coordination and critically reviewed the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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