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Research Article

Preliminary Assessment of Secondary Metabolites of Selected Pteridophytes from Jorhat, Assam, India

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ABSTRACT

This study analyses the phytochemical constituents of seven pteridophytic species from Assam, India. The methanolic extracts of seven species: *Pyrrosia lanceolata*, *Pyrrosia nummularifolia*, *Ampheneuron opulentum*, *Sphenomeris chinensis*, *Selaginella* sp., *Diplazium esculentum*, and *Blechnum orientale* were screened for phenolic, flavonoid, and flavonol content using standard spectrophotometric methods. *Pyrrosia nummularifolia* exhibited the highest flavonoid content, while *Diplazium esculentum* had the highest flavonol concentration. These bioactive compounds, known for their antimicrobial, antioxidant, and anti-inflammatory properties, support their traditional medicinal use and highlight their potential for drug discovery. Further studies are required to isolate and identify the pharmacologically active compounds from these species.

Keywords: Flavonoids; flavanols; phytochemical analysis; phenolic content; pteridophytes; traditional medicine.

INTRODUCTION

Pteridophytes, to which ferns belong, are among the earliest vascular plant groups, originating millions of years ago. These non-flowering vascular plants have played an essential role in plant evolution and ecology. Pteridophytes exhibit resistance to microbial infections, which may have contributed to their evolutionary success and longevity of over 350 million years (Sharma & Vyas, 1985). This resistance is attributed to a wide range of phytochemical constituents that play crucial roles in defence, reproduction, and growth. Essential compounds such as polysaccharides, proteins, carbohydrates, amino acids, and nucleic acids are primary metabolites that directly support plant growth and metabolism. In addition to primary metabolites, plants also produce secondary metabolites, which act as signalling molecules within the cell and are not essential for growth, development, or reproduction. Secondary metabolites, such as flavonoids, alkaloids and phenols, are synthesized through distinct metabolic pathways that diverge from the primary ones (Handique, 2024). Flavonoids, for instance, are stored in the vacuoles of plant cells as water-soluble pigments and have a fundamental C6-C3-C6 carbon framework consisting of two 6-carbon benzene rings.

With increasing research on phytochemical constituents, many pteridophyte species have been incorporated into the pharmacopoeia of various countries (Ibadullayeva et al., 2022). Among the diverse range of secondary metabolites, terpenoids are among the most prevalent bioactive compounds in ferns (Ho et al., 2010). These bioactive compounds possess a broad spectrum of medicinal properties, including anti-inflammatory, antioxidant, anti-tumour, antimicrobial, antiviral, and anti-HIV activities, and are generally regarded as safe (Proestos et al., 2005). Flavonoids, terpenoids and other secondary metabolites play a crucial role in pharmaceutical research due to their wide range of biological activities. Flavonoids, for instance, are well documented for their antioxidant, anti-inflammatory, and antimicrobial properties, making these potential candidates for drug formulations targeting oxidative stress-related diseases, infections and inflammation (Proestos et al., 2005).

Terpenoids are among the most structurally diverse classes of secondary metabolites in ferns and have demonstrated significant pharmacological potential. Some terpenoids have been shown to exhibit anticancer properties by inducing apoptosis and inhibiting tumour progression, while others function as potent antiviral or antibacterial agents (Ho et al., 2010). In recent studies, specific terpenoids from *Blechnum orientale* have displayed strong cytotoxic effects against cancer cell lines, highlighting their potential in chemotherapeutic drug development (Raja & Paul, 2019). The ability of these compounds to interact with key cellular pathways and enzymes has made them attractive candidates for new drug discovery programmes.

Therefore, this study aims to assess the antioxidant capacity of seven fern species—*Pyrrosia lanceolata*, *Pyrrosia nummularifolia*, *Ampheneuron opulentum*, *Sphenomeris chinensis*, *Selaginella* sp., *Diplazium esculentum*, and *Blechnum orientale*—collected from Assam, Northeast India. These species were selected for their traditional medicinal use and ecological relevance. This research seeks to contribute to the limited literature on fern phytochemistry and explore the pharmacological potential of these species.

Despite their widespread distribution, research on the phytochemical properties of pteridophytes remains limited. Many species contain bioactive compounds with medicinal properties, including flavonoids, alkaloids, and phenols, which contribute to their pharmacological potential. However, the specific phytochemical composition of several species from Northeast India remain unexplored. The selection of these seven species was based on their traditional medicinal uses and ecological significance in Assam. Thus, further exploration of these bioactive compounds, including their structural elucidation and bioassay-guided fractionation, is necessary to determine their full pharmacological potential and applicability in modern medicine**.**

METHODOLOGY

Collection of plant materials

Fresh fern specimens were collected from the Jorhat District of Assam, India (26.7509° N, 94.2037° E) in August, 2024 (Fig. 1). The specimens were authenticated and identified by the Department of Botany, Jagannath Barooah University, Jorhat, Assam, India (Table 1).

Phytochemical screening

Preliminary phytochemical screening of the fern extracts was conducted following the standard protocol described by Handique (2024).

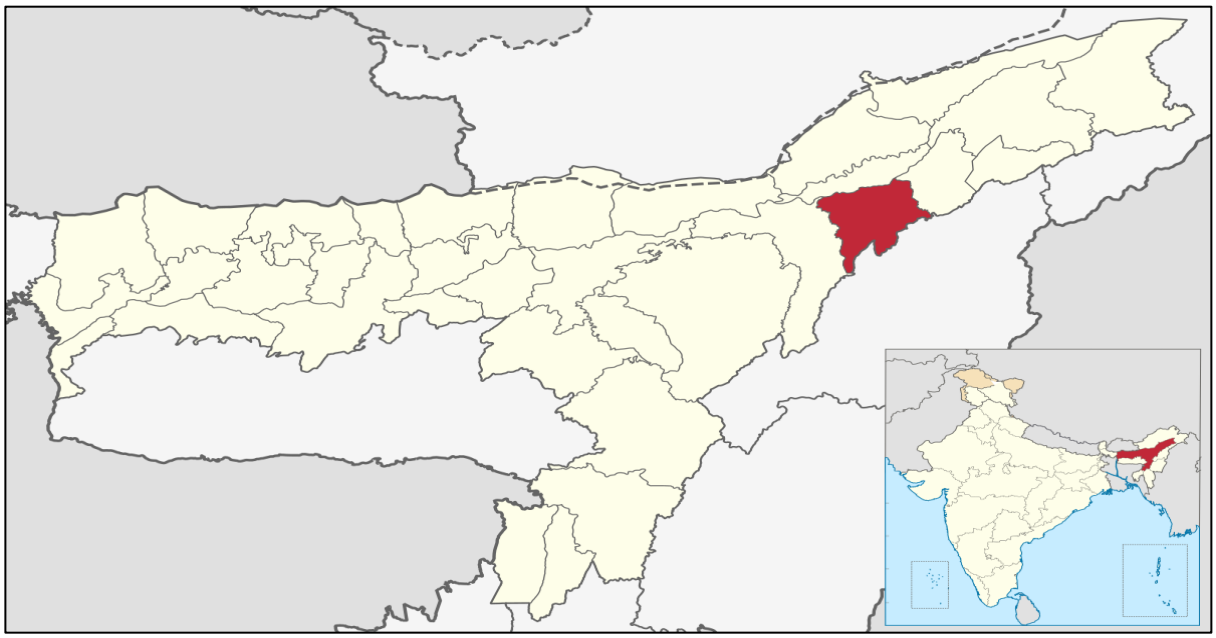

Figure 1: Map of Assam (Study area with Jorhat district highlighted).

Table 1: Collection Sites and GPS Coordinates of Selected Pteridophyte Species from Jorhat, Assam, India.

Species name	Location	GPS coordinates
Pyrrosia lanceolata	J.B. College, Jorhat	26.7506°N, 94.2033°E
Pyrrosia nummularifolia	Lachit Nagar, Jorhat	26.7461°N, 94.2175°E
Ampheneuron opulentum	Kaliapani Reserve Forest	26.7350°N, 94.2985°E
Sphenomeris chinensis	Rajabari, Jorhat	26.7520°N, 94.2194°E
Selaginella sp.	Borbheta, Jorhat	26.7254°N, 94.1937°E
Diplazium esculentum	J.B. College, Jorhat	26.7506°N, 94.2033°E
Blechnum orientale	Kenduguri, Jorhat	26.7602°N, 94.2221°E

Preparation of plant extract

An electronic balance was used to accurately weigh 0.5 g of dried fern leaves. The plant material was then transferred into a mortar, and 50 mL of 90% methanol (Merck, India) was added. Methanol was selected as the extraction solvent due to its high polarity and effectiveness in dissolving a broad range of bioactive compounds such as phenols and flavonoids (Harborne, 1998; Senguttuvan et al., 2014). The fern leaves were macerated thoroughly using a pestle to ensure complete cellular disruption and maximum extraction of secondary metabolites.

Drying and filtration

The macerated mixture was transferred to a clean glass beaker and placed in a hot air oven at 40 °C for 20 minutes under monitored conditions in a well-ventilated laboratory environment to facilitate partial evaporation of methanol. Following this, the extract was filtered using Whatman No.1 filter paper or equivalent (HiMedia, India) to remove insoluble plant residue. The resulting crude fern extract was collected and stored in sterile, labeled containers for further phytochemical analyses.

All experiments were conducted in triplicate (n = 3) to ensure reproducibility and accuracy. For each fern species, five individual specimens were analyzed, and extractions were performed independently on three biological replicates per specimen.

Test for phenol

The total phenolic content (TPC) of the fern extracts was estimated using the Folin–Ciocalteu method, originally described by Singleton and Rossi (1965), with slight modifications reported by Ainsworth and Gillespie (2007) and Adusei et al., (2019) was used. In this procedure, 0.5 mL of Folin–Ciocalteu reagent (HiMedia, India), pre-diluted 1:1 with double-distilled water, was added to 0.5 mL of fern extract in a test tube. The mixture was gently shaken and incubated at room temperature (25 ± 2 °C) for 5 minutes.

Next, 2 mL of 2% sodium carbonate (Na_2CO_3; SRL, India) solution (prepared by dissolving 2g of Na_2CO_3 in 100 mL of distilled water) was added, and the tubes were incubated in the dark for 10 minutes to allow the blue chromophore to develop. Absorbance was measured at 730 nm using a UV-Visible spectrophotometer (Thermo Scientific Evolution 201). A standard calibration curve was prepared using gallic acid (SRL, India). Results were expressed as milligrams of gallic acid equivalents per gram of dry fern extract (mg GAE/g).

Test for flavonoids

The total flavonoid content (TFC) of the fern extracts was determined using the aluminum chloride colorimetric method, following the protocols of Goyal et al., (2010), with slight modifications reported by Seifu et al., (2017) and Ayele et al., (2022). In short, a 10 mL test tube with 2 mL of distilled water was filled with an aliquot (0.5 mL) of the extract. 0.15 mL of 5% sodium nitrite ($NaNO_2$, SRL, India) solution (prepared by dissolving 5 g of $NaNO_2$ in 100 mL of double-distilled water) was added to each test tube. After incubating for five minutes, 0.15 mL of 10% aluminium chloride, ($AlCl_3$) solution (prepared by dissolving 10 g of $AlCl_3$ (SRL, India) in 100 mL distilled water) was added. 1 mL of 1 M sodium hydroxide (NaOH, Merck, India) solution (prepared by dissolving 4 g of NaOH pellets in 100 mL distilled water) was added after 1 minute, and the volume was then adjusted with distilled water to reach 5 mL. The absorbance of the resultant solution was measured using a UV-Visible spectrophotometer (Thermo Scientific Evolution 201) at 510 nm after 10 minutes. To express the total flavonoid content of samples as mg quercetin equivalent per 100 g of sample (mg QE/100 g sample), catechin was used as a standard. Three separate analyses were performed on each sample.

Test for flavanols

The total flavonol concentration was quantified using the aluminum chloride colorimetric method, following the protocol of Kumaran and Karunakaran (2006) with slight modifications reported by Handique, (2024). Briefly, 2 mL of fern extract was mixed with 2% aluminium chloride ($AlCl_3$; HiMedia, India) and 5% sodium acetate (CH_3COONa) (prepared by dissolving 0.41 g of sodium acetate in 100 mL of distilled water). The mixture was incubated at room temperature (approximately 25°C) for 2.5 hours. Absorbance was then measured at 440 nm using a UV-visible spectrophotometer (Thermo Scientific Evolution 201).

Statistical analysis

All experiments were conducted in triplicates, and results are expressed as mean ± standard deviation (SD). SD was calculated using Microsoft Excel based on three independent measurements for each species. One-way ANOVA followed by Tukey's post hoc test was performed to assess significant differences ($p < 0.05$) among species.

RESULTS

A preliminary survey of the study area revealed a high diversity of pteridophytes belonging to various families. A checklist of the recorded pteridophytes species is provided in Table 2.

Table 2: A checklist of pteridophytes found in Jorhat district, Assam, India.

Sl No.	Species	Family	Habit	Reference
1	*Salvinia cucullata* Roxb. Ex Bory	Salviniaceae	Aquatic herb	(Borthakur et al., 2000)
2	*Diplazium esculentum* (Retz.) Sw.	Athyriaceae	Herb	(Borgohain & Hazarika, 2020)
3	*Lygodium japonicum* (Thunb.) Sw.	Schizaceae	Climber	
4	*Pyrrosia lanceolata* (L.) Farw.	Polypodiaceae	Climber	
5	*Pyrrosia nummularifolia* (Sw.) Ching	Polypodiaceae	Climber	
6	*Ampheneuron opulentum* (Kaulf.) Holttum	Thelypteridaceae	Terrestrial Herb	
7	*Odontosoria chinensis* (L.) J.Sm.	Lindsaeaceae	Terrestrial Herb	
8	*Selaginella* sp.	Selaginellaceae	Terrestrial Herb	
9	*Blechnum orientale* L.	Blechnaceae	Terrestrial Herb	
10	*Thelypteris namburensis* (Bedd.) C.F.Reed	Thelypteridaceae	Terrestrial Herb	(Chandra et al., 2008)
11	*Azolla pinnata* R.Br.	Salviniaceae	Aquatic Herb	(Gogoi et al., 2023)
12	*Marsilea quadrifolia* L.	Marsileaceae	Aquatic Herb	(Gogoi et al., 2023)
13	*Stenochlaena palustris* (Burm.) Bedd.	Blechnaceae	Climber	(Dutta et al., 2017)

Phytochemical analysis of the methanolic extracts from the seven fern species revealed the presence of key secondary metabolites: phenols, flavonoids, and flavanols. *Pyrrosia lanceolata*, *Pyrrosia nummularifolia*, *Diplazium esculentum* and *Blechnum orientale* (0.331 mg/g) exhibited the highest phenol content, while *Sphenomeris chinensis* displayed the lowest (0.042 mg/g).

Flavonoid content varied among the species, with *Pyrrosia nummularifolia* having the highest level (1.732 mg/g) and *Pyrrosia lanceolata* the lowest (0.322 mg/g). For total flavanol content, *Diplazium esculentum* contained the maximum amount (8.039 mg/g), while *Pyrrosia lanceolata* (4.327 mg/g) had the minimum. These findings highlight the presence of bioactive compounds with potential medicinal properties in the fern species examined (Table 3). The identified phytochemicals have previously been associated with medicinal and physiological activities, supporting their use in the treatment of various ailments (Fig. 2, 3 and 4).

Table 3: Phytochemical Composition (Phenol, Flavonoid, and Flavonol Content) of the Studied Pteridophytes (Mean ± SD, n = 3).

Specimen	Phenol Content (mg GAE/g)	Flavonoid Content (mg QE/g)	Flavonol Content (mg RE/g)
Pyrrosia lanceolata	0.331 ± 0.007	0.322 ± 0.004	4.327 ± 0.020
Pyrrosia nummularifolia	0.331 ± 0.003	1.730 ± 0.006	7.500 ± 0.024
Ampheneuron opulentum	0.086 ± 0.002	0.487 ± 0.002	5.636 ± 0.015
Sphenomeris chinensis	0.042 ± 0.001	0.638 ± 0.003	7.250 ± 0.021
Selaginella sp.	0.281 ± 0.003	0.507 ± 0.005	5.145 ± 0.017
Diplazium esculentum	0.331 ± 0.003	0.388 ± 0.003	8.038 ± 0.028
Blechnum orientale	0.331 ± 0.004	0.572 ± 0.005	6.676 ± 0.023

One-way ANOVA showed a statistically significant difference in phenol, flavonoid, and flavanol content among species ($F = 116.65$, $p < 0.05$). However, Tukey's post hoc test did not detect significant pairwise differences ($p > 0.05$ for all comparisons), indicating that while species differ overall, no two species had significantly different flavonoid content at the 95% confidence level.

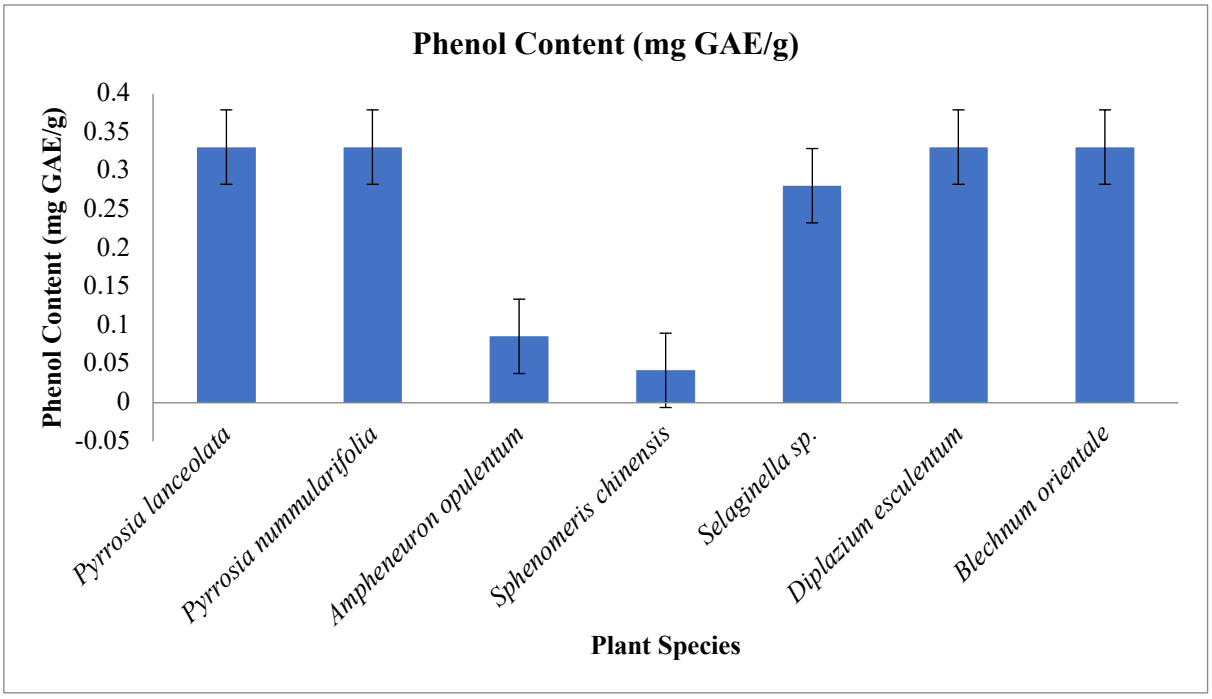

Figure 2: Bar Graph Showing phenol content (mg GAE/g) in the pteridophytic species.

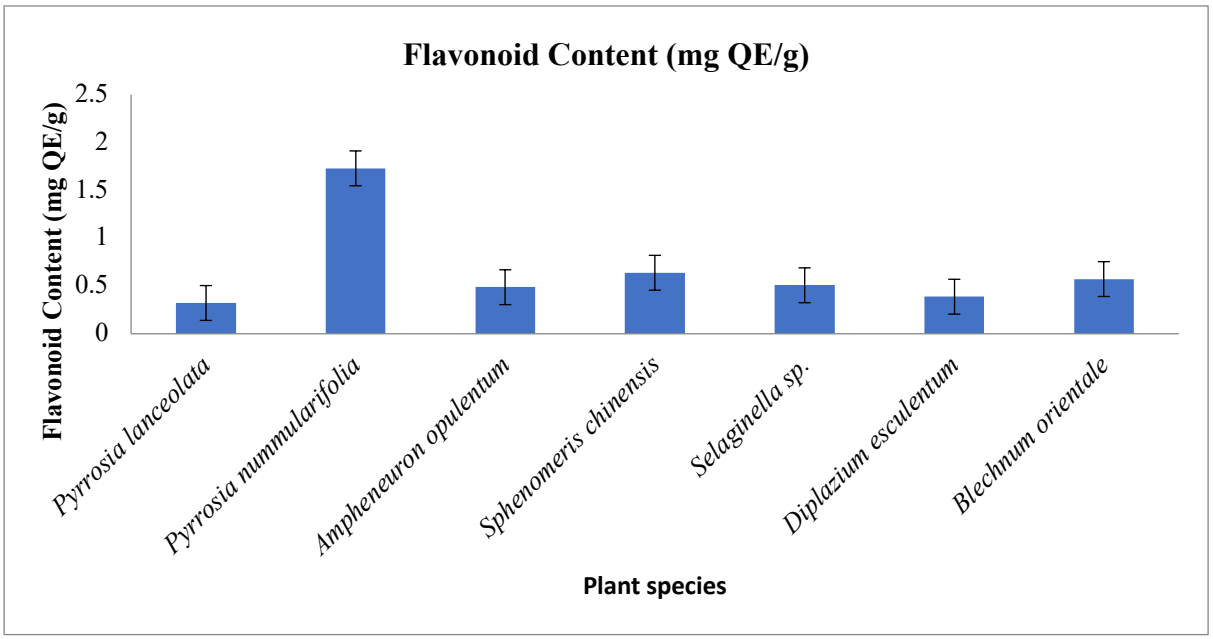

Figure 3: Bar Graph Showing flavonoid content (mg QE/g) in the pteridophytic species

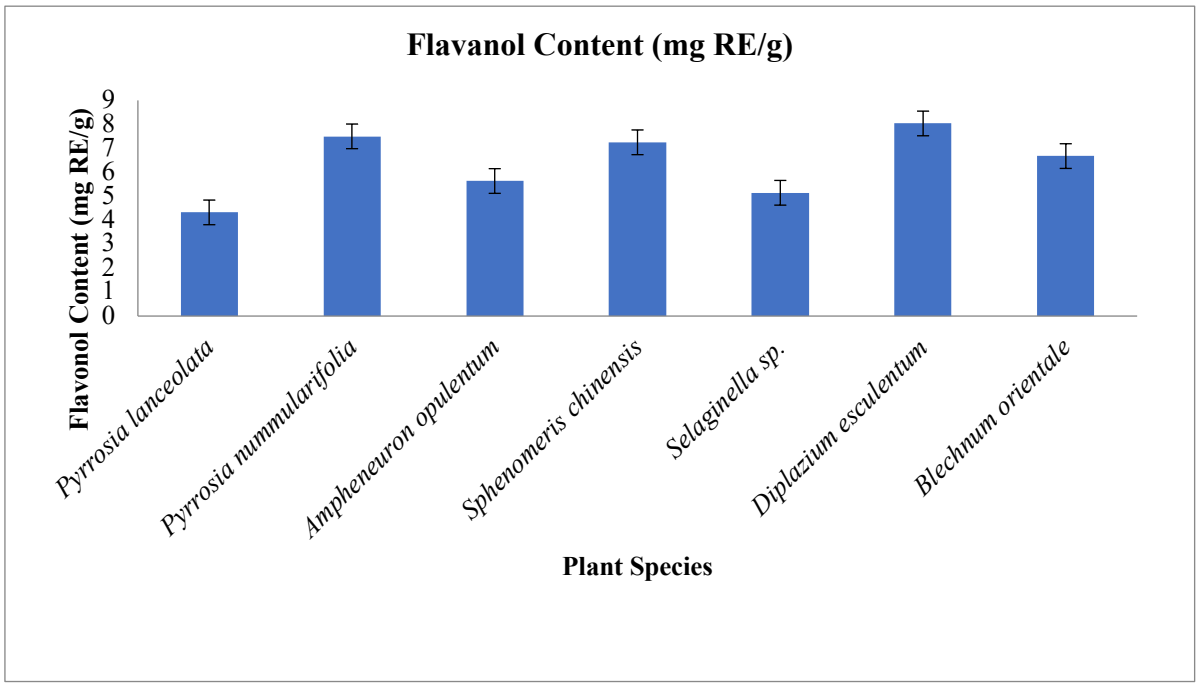

Figure 4: Bar Graph Showing flavanol content (mg RE/g) in the pteridophytic species

DISCUSSION

A study conducted in Northeast India revealed that methanol extracts from three plant species contained the highest concentration of phytoconstituents, suggesting their unique physiological properties could contribute to drug discovery. Notably, *Pyrrosia lanceolata* extracts, particularly the ethyl acetate extract, exhibited strong antioxidant properties, comparable to

ascorbic acid. These benefits are likely attributed to flavonoids, a class of phenolic compounds known for their ability to neutralize free radicals (Indriani et al., 2023).

Phenolic compounds, including flavonoids, are widely recognized for their pharmacological significance, particularly their antioxidant, antimicrobial, and anti-inflammatory properties. Species with higher phenolic content, such as *Diplazium esculentum* (8.038 mg/g) and *Pyrrosia nummularifolia* (7.500 mg/g), are associated with strong antioxidant potential due to their phenolic constituents. Previous studies have reported antioxidant activity in *D. esculentum*, supporting its traditional medicinal use (Choudhury et al., 2017). Additionally, flavonoid-rich species, including *P. nummularifolia* (1.730 mg/g), have been linked to antimicrobial and anti-inflammatory effects, which may contribute to their application in wound healing and infection management. These findings reinforce the therapeutic potential of these species and provide scientific validation for their continued use in herbal medicine.

Ismiarni et al., (2015) investigated the anti-inflammatory activity of *Nephrolepsis falcata* and *Pyrrosia lanceolata*, confirming their significant anti-inflammatory effects. Fan et al., (2020) analyzed six *Pyrrosia* species and identified the presence of phytol, nonanal, and 2,4-pentadienal, which exhibited broad-spectrum antibacterial activity. These findings align with the present study, further supporting the potential of *Pyrrosia lanceolata* as a therapeutic agent. Additionally, research on *Blechnum orientale L.* identified twelve secondary metabolites, including alkaloids, anthocyanins, anthraquinones, cardiac glycosides, diterpenes, flavonoids, saponins, steroids, tannins, and triterpenoids, alongside 20 compounds detected via GC-MS analysis (Raja & Paul, 2019). A separate phytochemical study on *B. orientale* confirmed the presence of various bioactive compounds, such as alkaloids, cardioglycosides, phenols, saponins, tannins, terpenoids, quinones, flavonoids, glycosides, anthocyanins, and betacyanins. Flavonoids, tannins, and phenolic substances were found in every fern species that was examined (Borkotoky et al., 2024). *P. semipinnata* extracts in ethanol and acetone produced the highest levels of flavonoids and tannins (302.73 ± 0.001 mg QE/g and 421.227 ± 0.009 mg GAE/g, respectively), as well as the maximum amount of extractable total phenolic components (526.517 ± 0.002 mg GAE/g and 526.517 ± 0.001 mg GAE/g, respectively).

Additionally, GC-MS analysis of ethyl acetate and ethanol extracts identified several antibacterial compounds, further supporting the species' therapeutic potential (Amose et al., 2023). Choudhury et al., (2017) analysed the phytochemical constituents of *Diplazium esculentum*, identifying antioxidant properties and the presence of carbohydrates, saponins, phenols, flavonoids, proteins, triterpenes, and alkaloids, but excluding glycosides, Phyto steroids, tannins, amino acids, and cardiac glycosides.

CONCLUSION

This study investigates the phytochemical composition of seven fern species from the Jorhat District of Assam, India, revealing the presence of phenols, flavonoids, and flavanols. These bioactive compounds, known for their antioxidant, antimicrobial, and anti-inflammatory properties, have traditionally been used in the treatment of wounds, cuts, tooth and gum pain, constipation, jaundice, digestive disorders, and cardiovascular ailments.

The findings highlight the potential of these species as novel therapeutic agents; however, further research is required to isolate, purify, and characterize the active compounds responsible for their medicinal effects. Additionally, comprehensive pharmacological and

toxicological studies are essential to validate their efficacy and safety for future drug development.

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DECLARATIONS

Research permit(s): Not applicable.
Ethical approval/statement: Not applicable.
Generative AI use: I/we declare that generative AI was not used in this study nor in the writing of this article.

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