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BIOACTIVE PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL OF *Lycopodiella cernua*: AN UNDEREXPLORED CLUBMOSS

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ABSTRACT

Lycopodiella cernua L. commonly known as clubmoss, is a medicinal pteridophyte with potential pharmaceutical applications. The present study aimed to evaluate the phytochemical constituents and antioxidant activity of *Lycopodiella cernua* collected from the Deomali Hills of Koraput District, Odisha, India. Plant specimens were collected, documented and authenticated through herbarium preparation following standard taxonomic procedures. Phytochemical analysis was conducted to quantify major bioactive compounds, including tannins, phenols and flavonoids. The results revealed the presence of appreciable amounts of tannins at 5.842 mg per 100 g, flavonoids at 1.865 mg per 100 g and phenols at 0.549 mg per 100 g, reflecting its richness in biologically active secondary metabolites. Antioxidant activity of the extract was assessed using standard *in vitro* methods and demonstrated notable free radical scavenging potential, supporting its ability to counter oxidative stress. The observed antioxidant activity may be attributed to the synergistic effects of phenolic and flavonoid compounds present in the plant. These findings revealed the therapeutic significance of *L. cernua* and support its potential as a natural source of antioxidant compounds for pharmaceutical and nutraceutical applications. Further studies on the isolation and characterization of specific bioactive constituents are recommended to validate its therapeutic potential.
Keywords **:** Clubmoss, antioxidant activity, phenols, flavonoids, tannins, Deomali hills.

Introduction

Lycopodiella cernua (Fig. 1) commonly known as the tassel fern or clubmoss, is a lycophyte belonging to the family Lycopodiaceae which is widely distributed in tropical and subtropical regions of Asia, Africa, Oceania and America where it commonly inhabits open grasslands, forest margins, wetlands and disturbed habitats (Zhao *et al*., 2012). As one of the ancient vascular plant lineages, the species represents an important component of plant biodiversity and has attracted considerable scientific interest due to its unique morphology, ecological significance and rich secondary metabolite profile (Porquis *et al*., 2018). Members of the family Lycopodiaceae are well known for producing characteristic Lycopodium alkaloids, many of which exhibit significant pharmacological activities, particularly acetylcholinesterase inhibitory properties. Studies on *L. cernua* have reported the presence of diverse alkaloids and other bioactive phytochemicals with promising antioxidant, anti-inflammatory, antimicrobial and cytotoxic activities (Ho *et al*., 2009; Zhao *et al*., 2012; Porquis *et al*., 2018). This perennial terrestrial lycophyte characterized by creeping rhizomes and erect, repeatedly dichotomously branched stems that may attain heights of 30-100 cm. The species bears numerous small, spirally arranged microphylls and produces terminal strobili containing sporangia. Its distinctive candelabra-like branching pattern and vigorous vegetative growth facilitate rapid colonization of open and disturbed habitats (Thomas, 1981). The species is widely distributed throughout tropical and subtropical regions and is commonly found in moist,

acidic soils, grasslands, forest clearings, roadside embankments and post-disturbance environments.

Fig. 1: *Lycopodiella cernua*, at Deomali Hills, Koraput

According to Brownsey and Perrie (2020), it is one of the most widespread members of the Lycopodiaceae and exhibits considerable ecological adaptability. Phytochemical investigations have revealed the presence of Lycopodium alkaloids, flavonoids, phenolics and other secondary metabolites. Several alkaloids isolated from this species have demonstrated notable acetylcholinesterase inhibitory activity, showing potential applications in the management of neurodegenerative disorders (Ho *et al*., 2009; Zhao *et al*., 2012). Furthermore, extracts of the species have shown significant antioxidant and anti-inflammatory properties along with moderate cytotoxic activity against selected cell lines (Porquis *et al*., 2018). Owing to its medicinal potential, ecological importance and evolutionary significance as an ancient vascular plant, *L. cernua* continues to be a valuable subject for phytochemical, pharmacological and conservation research. The present study focuses on the exploration of this underutilized plant species in its natural habitat. Specimens were collected, processed for herbarium preparation, and accurately identified. Further, phytochemical analyses and antioxidant activity assays were performed to assess its chemical constituents and potential therapeutic value.

Materials and Methods

During botanical explorations conducted in the Deomali Hills of Koraput District, Odisha, populations of *Lycopodiella cernua* were located in their natural habitat. Photographic documentation of the plant and its diagnostic features was undertaken in the field. Representative specimens were collected and processed following standard herbarium techniques for taxonomic verification. The species was identified with the help of regional floras (Saxena and Brahmam, 1994) and authenticated through comparison with digital herbarium resources and the Plants of the World Online database (POWO, 2026). The collected plant material was subsequently utilized for phytochemical evaluation and assessment of its antioxidant potential.

Quantitative Phytochemical Analysis

Quantitative estimation of major bioactive constituents was carried out using established analytical procedures. The concentrations of total phenols, tannins, saponins, and flavonoids were determined to evaluate the phytochemical richness of the species.

Determination of Total Phenolic Content

For phenolic estimation, 0.5 g of plant material from each of three replicates was homogenized with 60% methanol using a mortar and pestle. The extracts were centrifuged five times at 5000 rpm for 20 minutes. Aliquots (0.1 and 0.2 ml) of the extract were transferred into test tubes, while one tube served as the blank. The volume in each tube was adjusted to 1 ml with 60% methanol. Subsequently, 1 ml of 0.1 N HCl and 1 ml of sodium nitrite–molybdate reagent were added sequentially. The reaction mixture was diluted with 5 ml distilled water, followed by the addition of 2 ml of 1 N NaOH. After incubation for 20 minutes under dark conditions, absorbance was measured at 515 nm. Total phenolic content was quantified using a standard calibration curve (Swain and Hills, 1959).

Determination of Tannin Content

Tannin content was estimated using tannic acid as the reference standard. Different concentrations of tannic acid were prepared and treated with Folin reagent and sodium carbonate to generate a standard curve. For sample extraction, 5 g of plant material was boiled in 75 ml distilled water for 30 minutes and centrifuged at 2000 rpm for 20 minutes. The supernatant was collected and diluted to a known volume. An aliquot of the extract was mixed with distilled water, Folin reagent and 20% sodium carbonate solution. Following incubation in the dark, absorbance was recorded at 720 nm. Tannin

concentration was calculated from the standard calibration graph (Sadasivam and Manickam, 2009).

Determination of Total Flavonoid Content

Total flavonoids were estimated using the aluminium chloride colorimetric method with quercetin as the standard. An aliquot (100 µl) of aqueous plant extract was mixed with 100 µl of 5% sodium nitrite and allowed to react for 5 minutes. Thereafter, 150 µl aluminium chloride solution was added and incubated for 6 minutes. Subsequently, 200 µl of 1 M sodium hydroxide was introduced, and the reaction volume was adjusted to 3 ml with distilled water. The mixture was incubated for 15 minutes at room temperature under dark conditions. Absorbance was measured at 510 nm, and flavonoid concentration was determined using the quercetin standard curve (Chang *et al*., 2002; Shirazi *et al*., 2014; Raina and Misra, 2023).

Antioxidant Activity

Antioxidant activity was assessed using the DPPH free radical scavenging assay. Dried leaf powder (1 g) was extracted separately with distilled water and ethanol by maceration in 10 ml solvent for 48 hours with occasional shaking. The extracts were filtered, concentrated in a hot-air oven and dissolved in 1% Dimethyl Sulfoxide (DMSO) to obtain working solutions (Abubakar and Haque, 2020). A 0.1 mM DPPH solution was prepared in methanol and protected from light using aluminium foil (Baliyan *et al*., 2022). Different concentrations of the extracts were mixed with DPPH solution in a 1:1 ratio. Extract blanks (without DPPH) were used for background correction, while DPPH solution served as the control. After incubation in the dark for 10 minutes at room temperature, absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Radical scavenging activity was expressed as percentage inhibition and compared with the standard quercetin by plotting inhibition against extract concentration (Majhi *et al*., 2026).

$$\text{Inhibition} = \left[\frac{\text{Absorbance}_{(\text{control})} - \text{Absorbance}_{(\text{sample})}}{\text{Absorbance}_{(\text{control})}} \right] \times 100$$

Results and Discussion

Collection and herbarium preparation of *Lycopodiella cernua*

Healthy specimens of Lycopodiella cernua were collected from their natural habitat during field surveys. The collected plants exhibited characteristic morphological features including slender creeping stems, erect branched shoots, and densely arranged microphyllous leaves, which facilitated their identification. Photographs of the plant and its habitat were recorded during collection to support taxonomic documentation. The collected specimens were processed following standard herbarium techniques. Plant samples were carefully pressed, dried, mounted on herbarium sheets and labelled with relevant collection information. The prepared herbarium specimens retained important diagnostic characters necessary for accurate identification and long-term preservation (Fig. 2).

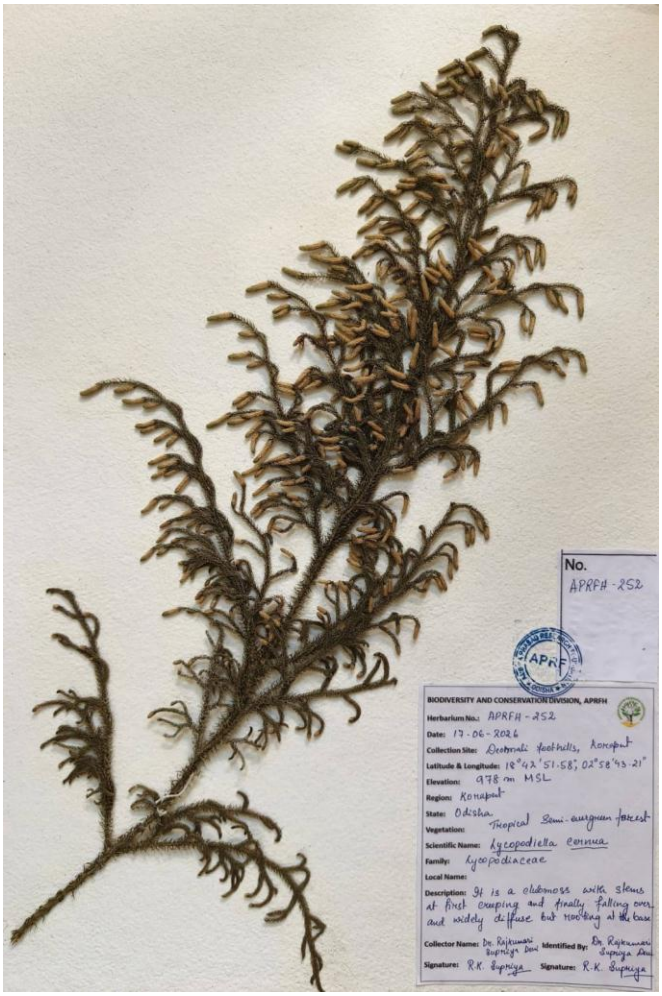

Fig. 2: Herbarium specimen of *Lycopodiella cernua*

Quantitative Phytochemical Analysis

The quantitative estimation of secondary metabolites in *Lycopodiella cernua* (stem and leaves) revealed the presence of tannins, phenols, and flavonoids (Fig. 3). Among the analyzed compounds, tannins were recorded in the highest concentration (5.842 mg/100 g), followed by flavonoids (1.865 mg/100 g) and phenols (0.549 mg/100 g). The predominance of tannins indicates that *L. cernua* is a rich source of tannin-based phytochemicals, which are known for their antioxidant, antimicrobial and

protective properties against herbivory and environmental stress. The presence of flavonoids further suggests potential antioxidant and free radical scavenging activities, as these compounds play an important role in reducing oxidative damage. Although the total phenolic content was comparatively lower, phenolic compounds also contribute to the overall antioxidant potential of plants through their ability to donate hydrogen atoms and neutralize reactive oxygen species.

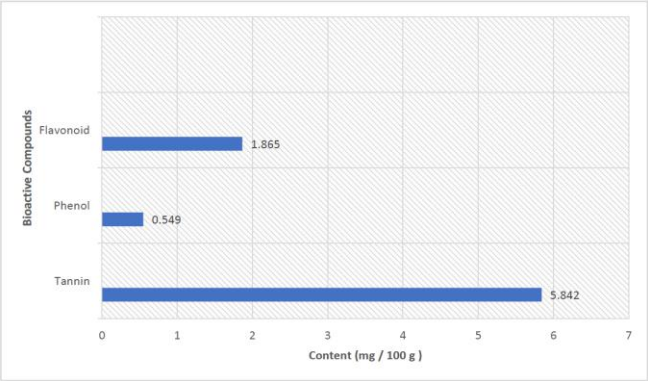

Fig. 3: Estimation of total tannin, phenol and flavonoids content in *L. cernua*

Antioxidant Activity

The antioxidant activity of aqueous, ethanol and chloroform extracts of *Lycopodiella cernua* was evaluated using the DPPH free radical scavenging assay. All extracts exhibited radical scavenging activity, with percentage inhibition increasing as extract concentration increased (Fig. 4 and 5). Among the tested extracts, the aqueous extract showed the highest antioxidant activity at all concentrations, with inhibition values increasing from 58.64% at 0.125 mg/ml to 77.76% at 1.0 mg/ml. The ethanol extract displayed moderate activity, ranging from 56.72% to 61.39%, while the chloroform extract exhibited the lowest activity, although its inhibition values also increased with concentration (54.80–61.39%). The superior performance of the aqueous extract revealed that water was more effective in extracting polar antioxidant compounds present in the stem and leaves of *L. cernua*. The standard antioxidant, quercetin, demonstrated markedly higher DPPH scavenging activity, with inhibition values ranging from 87.5% to 92.4% at concentrations of 5–20 µg/ml (Table 3, Graph 2). Although the plant extracts showed lower activity than quercetin, their considerable free radical scavenging potential confirms the presence of bioactive compounds capable of donating electrons or hydrogen atoms to neutralize DPPH radicals. The observed antioxidant activity may be attributed to the presence of secondary metabolites such as tannins, flavonoids, and phenolic compounds detected in the phytochemical analysis. These compounds are well known for their ability to scavenge reactive oxygen species and reduce oxidative stress. The findings indicate that *L. cernua* possesses appreciable antioxidant potential, particularly in the aqueous extract, and may serve as a promising natural source of antioxidant compounds for future pharmacological investigations.

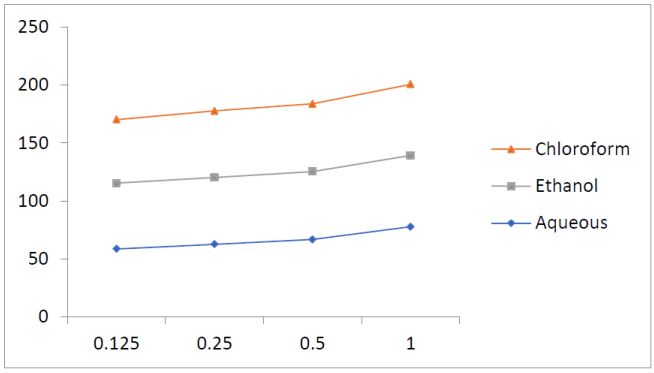

Fig. 4: DPPH free radical scavenging activity of different extracts of *Lycopodiella cernua*

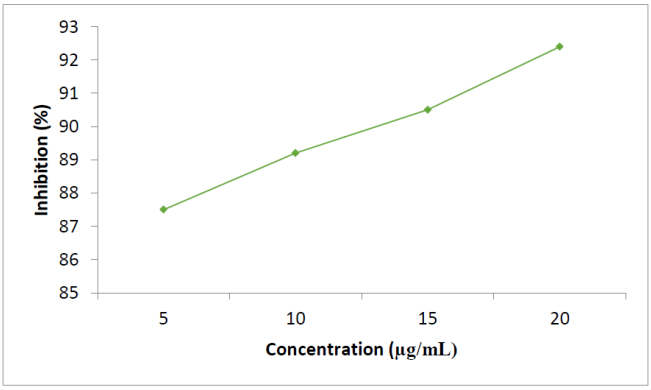

Fig. 5: DPPH free radical scavenging activity of standard (quercetin)

Conclusion

The present study demonstrated the systematic process of identification, herbarium preparation, collection of *L. cernua* and determining of phytochemical analysis revealed that it possesses appreciable phytochemical constituents and antioxidant potential. The presence of important secondary metabolites, including tannins, flavonoids and phenolic compounds with tannins being the predominant phytochemical might contribute to various biological activities, particularly antioxidant effects. Further studies involving isolation, characterization and pharmacological evaluation of its bioactive constituents are recommended to explore its therapeutic applications and validate its traditional uses.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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