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# PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EVALUATION OF HYDROETHANOLIC EXTRACT OF *LYCOPODIUM CLAVATUM* SPORES

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## ABSTRACT

*Lycopodium clavatum*, a club moss traditionally used in ethanomedicine, was investigated for its phytochemical constituents and antimicrobial potential. Preliminary phytochemical screening of hydroethanolic extract revealed the presence of alkaloids, flavonoids, terpenoids, carbohydrates and phenolic compounds. Antimicrobial activity was assessed using cup plate agar diffusion method against Gram-negative (*Escherichia coli)* bacterial strains. The hydroethanolic extract exhibited notable inhibitory zone, highlighting its board spectrum efficacy. These findings support the traditional use of *Lycopodium clavatum* spores and underscores its potential as a source of natural antimicrobial agents.

**KEYWORDS:** *Lycopodium clavatum,* phytochemical analysis, cup plate agar diffusion method, antibacterial activity.

## INTRODUCTION

Medicinal plants have long served as a cornerstone of traditional healing systems, offering rich bioactive compounds with therapeutic potential. Among these, *Lycopodium clavatum* (family Lycopodiaceae), commonly known as club moss, is a medicinal pteridophyte traditionally employed in homeopathy and folk medicine for treatment of digestive disorder,

rheumatism and cognative dysfunctions. Previous studies have reported that Lycopodium species are rich in alkaloids such as Lycopodine and Annotinine, which are known for their neuroprotective and anti-inflammatory activities.[1,2] Flavonoids present in various lycopodium extracts are associated with antioxidant properties. This study aims to analyse the phytochemical profile of *Lycopodium clavatum* extracts and assess their antimicrobial activity.

**Taxonomical classification**

- **Kingdom:** Plantae
- **Phylum:** Tracheophyta
- **Class:** Lycopodiopside
- **Order:** Lycopodiales
- **Family:** Lycopodiaceae
- **Genus:** Lycopodium
- **Species:** *Lycopodium clavatum*

**Distribution and Habitat**

Widely distributed across colder artic regions, temperate, tropical and subtropical regions. However most abundantly found in tropical zones. China, Turkey, Nepal, India (Arunachal Pradesh), North and South America, Jamaica, Japan, Korea. In India approximately thirty-three species of *Lycopodium* have been reported. These plants thrive in moist, shaded environments. Plants are pretty small, herbaceous usually have branched stem.[3,4] The leaves of plant are small and spores are green to yellow in colour.

**Figure 1: Lycopodium clavtum plant and spores.**

**Plant parts used,** whole plant, spore, aerial parts, leaves and stem.

**Chemical composition**

*Lycopodium clavatum* is known to contain a diverse array of bioactive compounds, with unique alkaloid profile. Lycopodium alkaloid are pharmacologically significant due to their distinctive chemical structures and biological activities. The studies have reported the presence of phenolic acids such as vanillic, coumaric, ferulic and syringic acids. Additionally, the plant also contains huperzine A, lycopodine, lycoflexine, α-onocerin and sporopollein.

A flavonoid polyphenol, apigenin has been isolated, which demonstrates potent antioxidant activity. Further studies identified lycopodine has the major alkaloid, along with clavatine and clavatoxine. The plant also contains polyphenolic acids such as dihydrocaffeic acid and triterpenes, contributing to its therapeutic potential.[5]

**Pharmacological activites**

The spores of *Lycopodium clavatum* are known to contain phytosterols, fatty acids, and estrified acids. Medicinally both aerial parts and spores of the plant are utilized for their diuretic and anti-inflammatory properties. Traditionally they have been employed in management of conditions such as Gout, Rheumatism, cancer[6,7] and Alzheimer's disease,[8] and are also widely used in Homeopathic formulations.[9] Alkaloids represent the major group of bioactive constituents in Lycopodium species.[10] These compounds have demonstrated therapeutic potential in treatment of cardiovascular and neuromuscular disorders, and are reported to enchance cognitive functions, including learning and memory.[11] Hepatoprotective, Immunomodulatory effects, antioxidant, Antiprotozoal, and pain and behavioural activity.[12]

**MATERIALS AND METHODS**

Dried spores of *Lycopodium clavatum* were extracted using hydroethanolic solvent (80% ethanol) through cold maceration.[13] The extract was concentrated and the percentage yield was determined. Preliminary phytochemical screening was carried out using standard qualitative methods to identify the presence of secondary metabolites and other phytoconstituents.

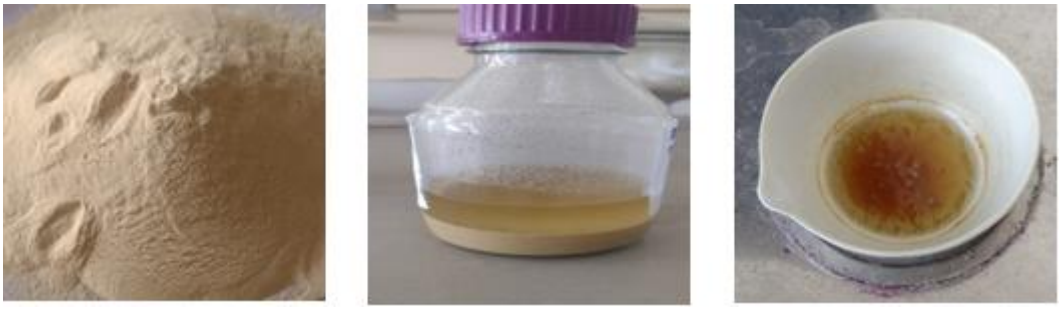

**Figure 2: Extraction of Lycopodium clavatum spores by maceration.**

**Preliminary qualitative phytochemical screening**[14]

**Test for carbohydrates**

Molisch's test: the extract mixed with small amount of Molisch's reagent (α-napthol dissolved in ethanol) in a test tube and small amount of concentrated sulphuric acid in the sides of the test tube. Appearance of a purple ring at the interface between two layers indicate presence of carbohydrates.

Barfoed's test: the extract mixed with small amount of barfoed's reagent (copper sulphate with acetic acid solution) and heated for few minutes. Appearance of brick red precipitate.

**Test for Alkaloids**

Mayer's test: small amount of extract is mixed with few drops of Mayer's reagent (mercuric chloride and potassium iodide in water) is added. White creamy precipitate is formed.

Dragondroff's test: small amount extract is taken in test tube and few drops of Dragondroffs reagent (potassium bismuth iodide solution) is added, which produces Orange/reddish brown precipitate.

Wagner's test: small amount of extract mixed with few d9rops of wagner's reagent (iodine and potassium iodide solution) is added. Reddish brown precipitate is formed.

**Test for Glycosides**

Modified Borntager's test: plant extract is mixed with few drops of ferric chloride solution and boiled for 5min cooled, equal volume of benzene is added and shaked well. The benzene layer is separated; few drops of ammonia solution is added. The appearance of rose pink coloured solution indicates the presence of glycosides.

**Test for Proteins and Amino acids**

Ninhydrin test: add few drops of 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Appearance of purple colour confirms the presence of proteins and amino acids.

Millon's test: add few drops of millon's reagent to the small quantity of extract. Apperaence of white precipitate indicates the presence of amino acids.

**Test for Tannins and Phenolic compounds**

Ferric chloride test: the small quantity of extract solution is mixed with few drops of 5% ferric chloride solution, produces dark blue or greenish black colour indicating the presence of tannins.

**Test for Flavonoids**

Alkaline reagent test: a small quantity of extract solution is mixed with 2mL of 2% sodium hydroxide solution and few drops of dilute HCl. An intense yellow colour is produced which becomes colourless on addition of dilute HCl. Confirms the presence of flavonoids.

Lead acetate test: Few mL of extract solution is mixed with few drops of 10% lead acetate solution. A yellow colour precipitate is produced indicating the presence of flavonoids.

**Test for Phytosterols**

Salkowski's test: few drops of extract is mixed with few drops of concentrated $H_2SO_4$ shaken well and allowed to stand, red colour appears in the lower layer confirming the presence of phytosterols.

**Antimicrobial activity**

Antimicrobial resistance (AMR) has emerged as a critical global health threat, the rapid evolution of resistant microbial strains has rendered many conventional therapies ineffective, necessitating the exploration of alternative treatment strategies. Medicinal plants contain a diverse group of alkaloids, phenols, flavonoids exhibit potent antimicrobial properties. Recent studies have demonstrated that combining plant-derived extracts with conventional antibiotics can produce synergistic effects, enhancing antimicrobial efficacy and potentially reversing resistance in multidrug- resistant strains.[15] In this study hydroethanolic extract of *Lycopodium clavatum* was evaluated using the agar well diffusion method. The assay was

designed to compare the inhibitory effects of the plant extract with a standard antibiotic (Amoxicillin) against a bacterial strain.[16]

**Procedure**

**Antibiotic sensitivity test,** conducted using the agar well diffusion method. Nutrient agar medium was prepared, and sterilized and 20mL was transferred into sterile culture tubes. After cooling to 40℃, 1mL of Bacterial inoculum was added to the medium and poured into sterile petri plates. Wells were bored aseptically using a sterile cork borer. Known volume of antibiotic and plant extract solutions were introduced into the wells. Plates were left at room temperature for 1hour to allow diffusion, followed by incubation at 37℃ for 18hours. Zone of inhibition were measured post-incubation to assess antibacterial activity.

**Preparation of test solution**

**Standard amoxicillin**

Equivalent to 30mg of amoxicillin powder was dissolved in 50mL of double- distilled water. Followed by the serial dilution with double distilled water to obtain final concentration of 50µg/ml.

**Plant extract (*Lycopodium clavatum)***

1g of extract was dissolved in 10mL of double distilled water (100mg/ml). serial dilutions were performed to obtain concentration of 1mg/ml, 10 mg/ml and 20mg/ml.

**RESULTS**

The percentage yield of the extract was found to 17%. Phytochemical screening results demonstrated in the table below;

| Sl no | Phytoconstituents | Present/absent |
|---|---|---|
| 1. | Carbohydrates | Present |
| 2. | Proteins | Absent |
| 3. | Alkaloids | Present |
| 4. | Glycosides | Absent |
| 5. | Tannins and phenols | Present |
| 6. | Flavonoids | Present |

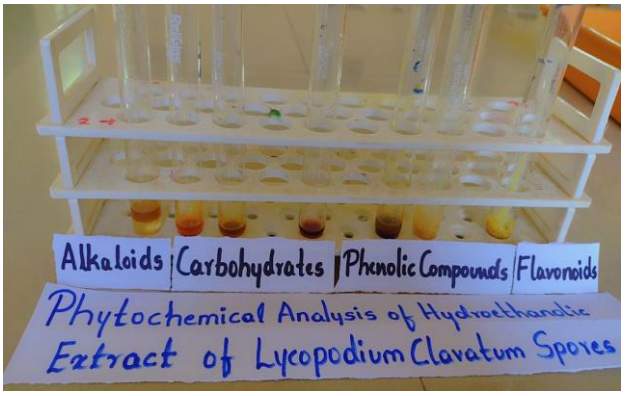


**Figure 3: phytochemical analysis of Lycopodium clavatum spores.**

The extract demonstrated notable antimicrobial activity, with visible inhibition of microbial growth at higher concentration with the most pronounced effects observed at 20mg/ml. When compared to standard, the test extract exhibited moderate activity, suggesting the presence of bioactive constituents.

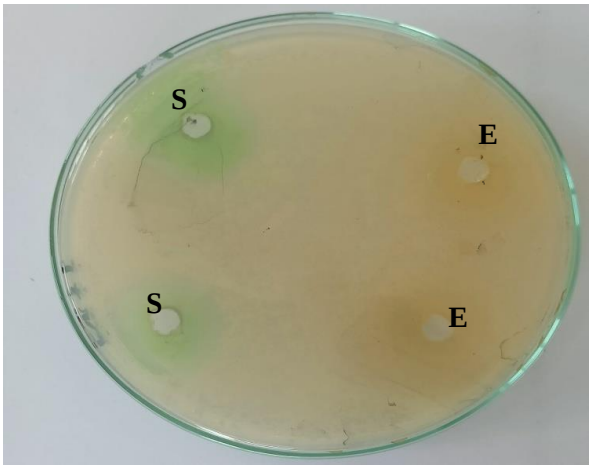


**Figure 4: ntimicrobial activity of Lycopodium clavatum spores S-Standard, E- Extract.**

## DISCUSSION

The presence of alkaloids in *Lycopodium clavatum* spores confirms earlier reports of Lycopodium alkaloids, which possess a wide range of pharmacological activities, including acetylcholinesterase inhibition and neuroprotection.[1,2] Flavonoids and tannins, detected in the extract, are well-documented antioxidants that may contribute to reducing oxidative stress in pathological conditions such as polycystic ovary syndrome (PCOS) and neurodegenerative diseases.[17,18] The absence of glycosides and proteins indicates that the spores are primarily enriched with secondary metabolites rather than storage compounds. In this study the Lycopodium extract showed inhibitory activity against E. coli showing its potent anti-microbial activity.

## CONCLUSION

Hydroethanolic extract of *Lycopodium clavatum* spores showed the presence of carbohydrates, alkaloids, tannins, phenols, and flavonoids and also exhibited a moderate anti-microbial activity These results highlight the medicinal potential of *Lycopodium clavatum* represent promising natural source for development of plant-based antimicrobial formulations. Further studies focusing on isolation, mechanism of action of its bioactive constituents and pharmacological activities are needed to be explored for full therapeutic potential.

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