Pharmacognostical and phytochemical evaluation of *Lycopodium clavatum* stem

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Paper presents pharmacognostical and phytochemical study of *L. clavatum*. Ferulic acid, a potential antioxidant present in this species, has been studied through HPTLC and may be utilized by industries for quality evaluation, ensuring successful commercial exploitation of this drug.

Key words: Ferulic acid, HPTLC, *Lycopodium clavatum*, Pharmacognosy, Phytochemical evaluation

**Introduction**

*Lycopodium* Linn. syn. *Huperzia* (Family: *Lycopodiaceae*), commonly known as “club moss, ground pine, devil’s claw, devil ash”, is a pteridophyte abundantly found in subtropical and tropical forests in the world. Many lycopods are used by tribals for memory-enhancing effect¹, against stomach pain², to treat burnt skin³, leaf decoction against muscle pain and rheumatism⁴, as a tonic or an analgesic to relieve rheumatic pain in joints and back⁵. *Lycopodium* Linn. has potent acetylcholinesterase inhibitory activity, as a future promising drug for treatment of Alzheimer’s disease⁶-⁹, for wound-healing effect against nappies occurring in babies and, therefore, also called “belly powder”¹⁰. Spores of the plant possess a protective effect as dusting powder for tender skin¹¹. Chemical investigations of lycopods have centered on alkaloids first discovered by Bodeker¹² and further by several workers¹³. *Lycopodium* species contained vanillic, p-coumaric and ferulic acids, in addition, to syringic acid¹⁴.

This paper presents pharmacognostical and phytochemical study of *L. clavatum* Linn. Ferulic acid, a potential antioxidant present in this species, has been studied through a simple and high-precision method using high performance thin layer chromatography (HPTLC).

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**Materials and Methods**

Plant material was collected from Kotayagiri of Nilgiri Hills, Ootacamund (Tamil Nadu), India. Material was authenticated, deposited in Institute’s Herbarium [LWG 221424, 2006] and stems were preserved in 70% ethyl alcohol for histological studies. Microtome sections were cut and stained with safranin and fast green and photographed with Nikon F70X camera¹⁵. Physicochemical and phytochemical studies¹⁶-¹⁸ were carried on the shade dried powdered material.

**HPTLC Studies**

Reagents used were from Merk (Germany) and standard ferulic acid was procured from Sigma-Aldrich (Steinheim, Germany). Air dried (45-55°C) powdered stem of *L. clavatum* (1.0 g) in triplicate were extracted separately with 3x10 ml methanol. Extracts were concentrated under vacuum, redissolved in methanol, filtered and finally made up to 100 ml with methanol prior to HPTLC analysis.

**Chromatographic Conditions**

Chromatography was performed on Merk HPTLC precoated silica gel 60GF²⁵⁴ (20x20 cm) plates. Methanolic solutions of samples and standard compound ferulic acid of known concentrations were applied to the layers as 6 mm-wide bands positioned 15 mm from the bottom and 15 mm from side of the plate, using Camag Linomat 5 automated TLC applicator with nitrogen flow providing a delivery speed of 150 nl/s from
Plate 1: Macroscopic & microscopic characters of *Lycopodium clavatum* stem: A) *Lycopodium clavatum* in natural habitat; B) Transverse section of stem diagrammatic view (x10); C) Transverse section of stem cellular view (x25); D) Transverse section of stem showing vascular region (x25); and E) Transverse section of stem cellular structure (x40) [Ep, Epidermis; Ed, Endodermis; Pc, Pericycle; Lt, Leaf traces; Co, Cortex; Mx, Metaxylem; Px, Protoxylem; Ph, Phloem]
application syringe. These conditions were kept constant throughout analysis of samples.

**Results**

**Macroscopic and Microscopic Characters of Stem**

Plants are pretty small, herbaceous (Plate 1A), usually have branched stems and small leaves, which are simple, small size and do not possess a ligule, and are arranged spirally around branched stem. Transverse section of *L. clavatum* stem shows a superficial epidermis, a broad cortex and a central massive vascular cylinder or stele (Plate 1 B & C). Epidermis is one cell in thickness, usually with thick, cutinized outer wall and stomata are present in epidermis. Cortex is quite broad and sclerenchymatous. There are three concentric zones in cortex; inner and outer zones are usually composed of elongated sclerenchymatous cells, with no inter cellular spaces and middle zone has large thin walled parenchymatous cell. In cortex, leaf traces are present. Endodermal cells show characteristic thickening on radial wall, known as *casperian strips*, within endodermis is pericycle composed of one or more layers of thin walled cells. Vascular system has xylem and phloem in alternative plates or bands and a stele of this type is known as plectostele (Plate 1D). Growth of both xylem and phloem is centripetal. Protoxylem is composed of simple reticulate elements. Metaxylem trachieds have circular bordered pits or scalariforms (transversely elongate) pits. Pitting is uniseriate to triseriate in trachieds, stele lacks a cambium and hence no secondary tissue is formed (Plate 1E).

**Physicochemical Studies**

Physicochemical and phytochemical studies (Fig. 1) of air dried plant material gave following values: moisture, 85; total ash, 7.85; acid insoluble ash, 1.23; alcohol soluble extractive, 4.25; water soluble extractive, 34.30; sugar, 17.64; starch, 27.86 and tannins, 23.35%.

**Detection and Quantification of Ferulic Acid**

Following sample application, layers were developed in a Camag twin trough glass chamber that had been presaturated with mobile phase of toluene: ethyl acetate: formaldehyde (6:3:1) till proper separation of bands up to 8 cm height. After development, layers were dried with a dryer and ferulic acid was simultaneously quantified using Camag TLC scanner model 3 equipped with Camag WinCATS IV software. Following scan conditions were applied: slit width, 6 mm x 0.45 mm; wavelength, 320
nm; and absorption-reflection mode. In order to prepare calibration curves, stock solution of ferulic acid (1 mg/ml each) was prepared and various volumes of these solutions were analyzed through HPTLC, calibration curves of peak area vs concentration were also prepared (Figs 2 & 3). Ferulic acid (yield, 0.443% dry basis) had following values: $R_f$, 0.61; and $r^2$, 0.986.

**Conclusions**

*L. clavatum* could be identified on the basis of presence of centripetal vascular bundle. Protoxylem is composed of simple reticulate elements; metaxylem trachieds have circular bordered pits or scalariforms (transversely elongate) pits. Pitting is uniseriate to triseriate in trachieds of *L. clavatum*. Stele lacks a
cambium and hence no secondary tissue is formed. HPTLC profile of this species showed presence of ferulic acid, which is not yet reported in this species.

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