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A HPTLC method for the identification of ferulic acid from *Lycopodium clavatum*

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ABSTRACT

Objective: To develop a simple precise and novel method for the identification and quantification of bioactive molecules ferulic acid in *Lycopodium clavatum*. **Methods:** Bioactive molecules ferulic acid in *Lycopodium clavatum* was indentified and estimated by the assay combined separation and quantitative estimation of the analyte on silica gel $60F_{254}$ HPTLC plates with visualization under UV and scanning at 320 nm. **Results:** HPTLC studies showed the separation and determination of ferulic acid (0.443%) in *Lycopodium clavatum*. The result of recovery ranged in 95%-97% for ferulic acid. **Conclusions:** This method is simple, sensitive, economic, novel and first time reported from this species. Therefore, ferulic acid might be a useful chemotaxonomic marker for the genus *Lycopodium* and other lycopods.

1. Introduction

Ferulic acid, a phenolic acid has wide distribution in the plant kingdom and is more bioavailable than other dietary flavonoid and monophenolics studied[1]. It has been proved to be a potent antioxidant, anti-inflammatory, and is reported to terminate free radical chain reaction and reduce the risk for coronary heart diseases[2-6].

Chemical investigations of the lycopods have centered on the alkaloids first discovered by Bodeker[7] and further by several workers[8-16]. Although these botanically interesting plants, of which there are possibly 483 species, are common elements of the flora of many parts of the world, including Europe and North America, they have not been examined extensively from either a biochemical or a chemical standpoint.

It is therefore of interest and has prompted us to study, in more detail, the ferulic acid content of *Lycopodium clavatum*. In view of its great importance worldwide, a simple and high-precision method has been developed to estimate ferulic acid using high performance thin layer chromatography (HPTLC) in this plant, which is not yet isolated by any researcher and will be utilized for chemotaxonomic significance of lycopods.

2. Materials and methods

2.1. Plant Material

The plant sample of *Lycopodium clavatum* (Family: Lycopodiaceae) was collected from the Kotayagiri of Nilgiri Hills, Ootacamund (Tamilnadu), India in the month of January, 2006. Authenticated specimens were deposited in the Institute's herbarium (LWG 221424, 2006).

2.2. Chemicals and reference marker compound

Reagents used were from Merck (Germany) and standards *viz.* ferulic acid was from Sigma-Aldrich (Steinheim, Germany) supplied through their Indian authorized agents at Lucknow, India.

2.3. Extraction of plant material for analysis

Air dried (45-55 ℃) powdered whole plant sample of *Lycopodium calavtum* (1.0 g) in triplicate were extracted separately with 3×10 mL methanol. Extracts were concentrated under vacuum, redissolved in methanol, filtered and finally made up to 100 mL volume with

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methanol prior to HPTLC analysis.

2.4. Chromatographic conditions

Chromatography was performed on Merck HPTLC precoated silica gel 60F_{254} (10 cm ×10 cm) plates (Cataloge No. 818133). 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 the nitrogen flow providing a delivery speed of 150 nL/s from the application syringe. These conditions were kept constant throughout the analysis of the samples.

2.5. Detection and quantification of the ferulic acid

Following sample application, layers were developed in a Camag twin through glass chamber that had been pre-saturated with the mobile phase of Toluene: Ethyl acetate: Formaldehyde (6:3:1), then the plate was developed to 8 cm height from the origin. After development, the layer was dried with a hair dryer and ferulic acid was simultaneously quantified using Camag TLC scanner model 3 equipped with Camag Wincats IV software. The following scan conditions were applied: silt width 6 mm ×0.45 mm; wavelength, 320 nm; absorption-reflection mode. In order to prepare calibration curves, stock solution of ferulic acid (1 mg/mL each) were prepared and various volumes of these solutions were analyzed through HPTLC exactly as mentioned above, calibration curves of peak area *vs.* concentration were also prepared.

2.6. Validation

The precision of the scanner was checked by scanning the same spots five times and the coefficient of the variance was calculated. The repeatability of the method was also established by applying 5 mg per spot of each standard solution five times and the coefficient of variance was calculated. The limit of detection (LOD) and limit of quantification (LOQ) was also determined.

3. Results

Different compositions of the mobile phase for HPTLC analysis were tried in order to optimize the method to obtain high resolution and reproducible peaks. The desired profile was achieved in the mobile phase of toluene: ethyl acetate: formaldehyde (6:3:1) at wavelength of 320 nm (Figure 1-3). Calibration curves for ferulic acid were linear within range of 1 μg to 10 μg. Ferulic acid was 0.443% dry weight, and R_f value was 0.61. Regression equation was y = 1 2269.781 x + 3 221.118, r^2=0.986. The method has been properly validated for instrument precision, repeatability, LOD, LOQ, specificity and linear regression (Table 1). Linearity of the calibration graph was tested by plotting residuals against quantities tested residuals are distributed at random around the zero line without any trend the calibration function can therefore be considered as linear. The purity of the standard was ascertained by checking the absorption spectra at the start, middle and end of the bands. The accuracy of recovery rate was also checked by analyzing plant samples in triplicate spiked with four different concentrations of stock solution of ferulic acid. The result of recovery ranges in 95%-97% for ferulic acid.

Table 1.
Validation data for the HPTLC method for the estimation of ferulic acid.

Property	Values
R_f	0.610
Instrumental precision (CV, n=5)	0.030
Repeatability (CV, n=5)	0.023
Limit of detection	1.143 μg
Limit of quantification	4.464 μg
Linear regression	0.986
Calibration range	1 μg to 10 μg
Specificity	Specific
Robustness	Robust

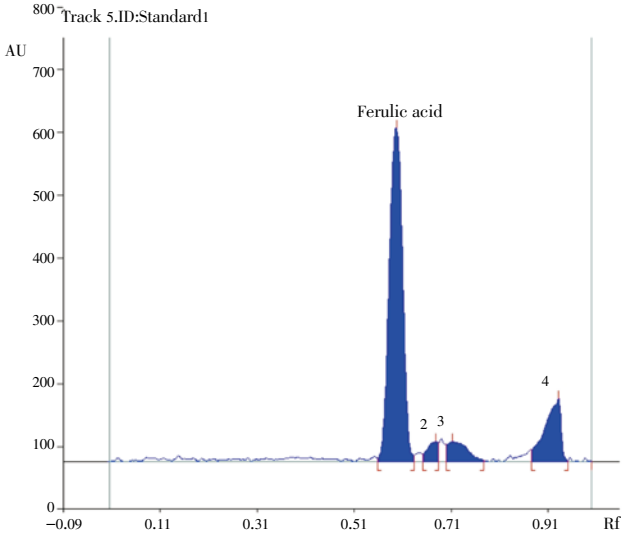

Figure 1. HPTLC densitometric scan (at 320 nm) of ferulic acid.

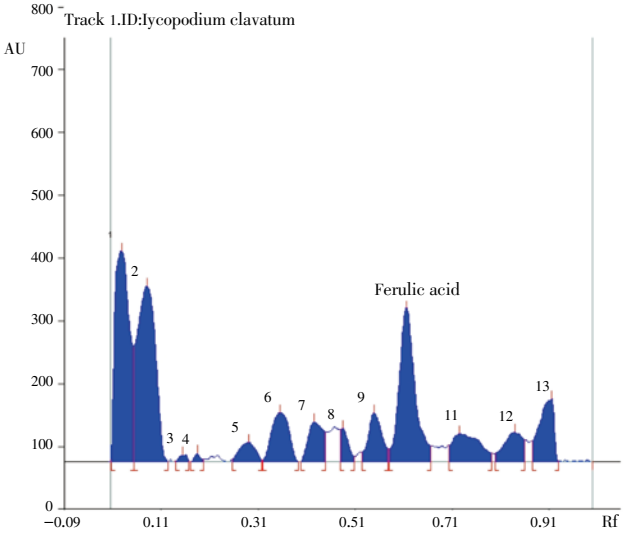

Figure 2. HPTLC densitometric scan (at 320 nm) of *Lycopodium clavatum* sample.

For the quantitative estimation of ferulic acid, analysis of sample was performed repeatedly three times. Average content of ferulic acid in the species are given in Table 1.

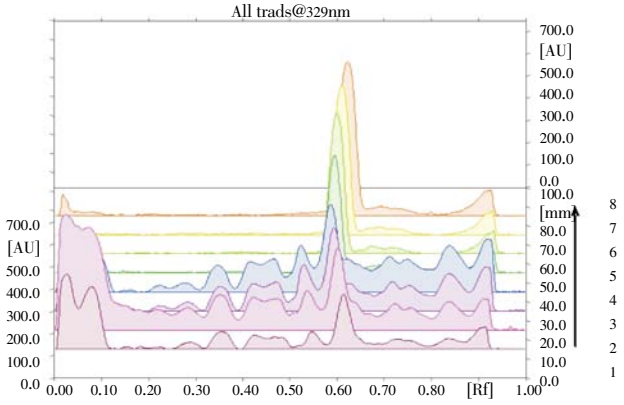

Figure 3. HPTLC chromatograms of *Lycopodium clavatum* samples (1-4) & Reference compound ferulic acid (5-8).
Lycopodium clavatum samples-1:5 μg; 2:10 μg; 3:15 μg; 4:20 μg.
Ferulic acid - 5:5 μg; 6:10 μg; 7:15 μg; 8:20 μg.

4. Discussion

Ferulic acid is more bioavailable than other dietary flavonoid and monophenolics studied. It has been proved to be a potent antioxidant, anti-inflammatory, and is reported to terminate free radical chain reaction and reduce the risk for coronary heart diseases. *Lycopodium clavatum* is considered to be an important ingredient in Homeopathic medicine for several usages. HPTLC studies showed the separation and determination of ferulic acid (0.443%) in *Lycopodium clavatum* with recovery range of 95%-97%. This study is new and not yet reported in this species.

On the basis of aforesaid studies, it is concluded that the above analysis of ferulic acid from *Lycopodium clavatum* is simple, sensitive, economic, novel and first time reported from this species. Therefore, ferulic acid might be a useful biomarker for the chemical studies in genus *Lycopodium* and other lycopods. Quantitative estimation of ferulic acid opens new vistas for this genus for its successful commercial exploitation in dietary suppliments.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Graf E. Antioxidant potential of ferulic acid. *Free Radic Biol Med* 2000; **28**: 1249-1256.
[2] Bourne LC, Panganga G, Baxter D. Absorption of ferulic acid from low alcohol beer. *Free Radic Res* 2000; **32**: 273-280.
[3] Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H. Antioxidant properties of ferulic acid and its related compounds. *J Agri Food Chem* 2002; **50**: 2161-2168.
[4] Ohta T, Nakano T, Egashira Y, Sanada H. Antioxidant activity of ferulic acid beta-glucuronide in the LDL oxidation system. Biosci *Biotechnol Biochem* 1997; **61**: 1942-1943.
[5] Orhan I, Küpeli E, Şener B, Yesilada E. Appraisal of anti-inflammatory potential of the clubmoss, *Lycopodium clavatum* L. *J Ethnopharmacol* 2007; **109**: 146-150.
[6] Özçelik B, Orhan I, Aslan S, Kartal M, Karaoglu T, Sener B, et al. P1109 Antimicrobial and antioxidant actions of the clubmoss *Lycopodium clavatum* L. *Intl J Antimicr Agents* 2007; **29**: S300
[7] Bodeker K. Justus Liebigs. *Ann Chem* 1881; **208**: 363.
[8] Ma X, Gang DR. The *Lycopodium* alkaloids. *Nat Prod Rep* 2004; **21**: 752-772.
[9] Choo CY, Hirasawa Y, Karimata C, Koyama K, Sekiguchi M, Kobayashi J,et al. Carinatumins A-C, new alkaloids from *Lycopodium carinatum* inhibiting acetylcholinesterase. *Bioorganic & Med Chem* 2007; **15**: 1703-1707.
[10] Ishiuchi K, Kubota T, Mikami Y, Obara Y, Nakahata N, Kobayashi J. Complanadines C and D, new dimeric alkaloids from *Lycopodium complanatum*. *Bioorganic & Med Chem* 2007; **15**: 413-417.
[11] Ishiuchi K, Kubota T, Morita H, Kobayashi J. Lycopladine A, a New C16N Alkaloid from *Lycopodium complanatum*. *Tetrahedron Lett* 2006; **47**: 3287-3289.
[12] Koyama K, Morita H, Hirasawa Y, Yoshinaga M, Hoshino T, Obara Y, et al. Lannotinidines A-G, New Alkaloids from Two Species of *Lycopodium*. *Tetrahedron* 2005; **61**: 3681-3690.
[13] Morita H, Ishiuchi K, Haganuma A, Hoshino T, Obara Y, Nakahata N, et al. Obscurumines A and B, new alkaloids from two Species of Lycopodium. *Tetrahedron* 2005; **61**: 1955-1960.
[14] Halldorsdottir ES, Jaroszewski JW, Olafsdottir ES. Acetylcholinesterase inhibitory activity of lycopodane-type alkaloids from the Icelandic *Lycopodium annotinum* ssp. *alpestre*. *Phytochemistry* 2010; **71**: 149-157.
[15] Konrath EL, Neves BM, Lunardi PS, Passos CS, Simões-Pires A, Ortega MG, et al. Investigation of the *in vitro* and *ex vivo* acetylcholinesterase and antioxidant activities of traditionally used *Lycopodium* species from South America on alkaloid extracts. *J Ethnopharmacol* 2012; **139**: 58-67.
[16] Ishiuchi K, Kubota T, Ishiyama H, Hayashi S, Shibata T, Kobayashi J. Lyconadins C and F, new *Lycopodium* alkaloids from *Lycopodium complanatum*. *Tetrahedron Lett* 2011; **52**: 289-292.