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HPLC AND HPTLC FINGERPRINT PROFILE OF ELYTRARIA IMBRICATA (VAHL) PERS.

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Abstract. The present study was aimed to develop the High performance thin layer chromatography (HPTLC) finger print profile of methanol extract of *Elytraria imbricata* belongs to the family Acanthaceae. Chromatographic technique was used for separation of components from extracts of this plant. This study was planned to develop a HPTLC fingerprint profile of methanol solvent extract. A HPTLC and HPLC method for the separation of the active constituents in extracts has been developed and TLC of these extracts on silica gel pre-coated aluminum plates of Merck by automatic TLC applicator and using solvent system toluene: ethyl acetate: methanol: hexane: formic acid (1.5:5.5:2:1:1) was performed. HPTLC finger print scanned at 200-400nm for methanol extract revealed 9 peaks with Rf values in the range of 0.9 to 0.74 respectively and HPLC finger print analysis of methanol extract revealed 4 peaks with Retention time value range from for 2.300 to 3.073 respectively. The HPTLC and HPLC method for routine quality control of present species can be carried out using this method for extract of this plant.

Keywords: *Elytraria imbricata*, Fingerprint, HPTLC, HPLC, Profile.

1. INTRODUCTION

Medicinal plants and herbal preparations have recently received considerable attention and have been found to be promising choice over modern medicines, in a number of studies. In developing countries, all over the world, 80% of population continues to use traditional medicine in primary medical problems.

HPLC method is gaining importance for qualitative and quantitative analysis of plant extracts, being useful for quality control of phytochemicals. This method is able to quantify the marker compounds in plants. This method enables the simultaneous identification of the major bioactive constituents present in medicinal herb.

HPTLC has emerged as a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs because of its simplicity, sensitivity, accuracy, suitability. High performance thin layer chromatography has emerged as one of the most efficient tools in the last two decades for the separation and quantification of secondary metabolites especially for the evaluation of botanical materials.

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Medicinal plants are a great source of economic values. Various plants and herbaceous plants used traditionally have potent antibacterial, antifungal and antiviral properties and such stories have called down the optimism about antimicrobial agents. Since antiquity, medicinal plants have been mapping a deep source of antimicrobial and antiviral agent.

2. Materials and Methods

2.1. **Plant Material.** Healthy plant *Elytraria imbricata* was collected from Yarcaud hills, Salem district in Eastern Ghats.

2.2. **Preparation of Plant Material.** In the present study the plant powder of *Elytraria imbricata* defatted with methanol. 100g was packed in a Soxhlet apparatus and extracted successively. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by Whatman filter paper. The extract was evaporated under reduced pressure using evaporator.

2.3. **HPTLC.**

2.3.1. **Sample preparation:** The methanol extract of each sample was used for HPTLC analysis. The extracts were filtered through a Whatman No.1 filter paper. 25 ml filtered extract was evaporated by a rotatory vaccum evaporator. 10 ml evaporated extracts were used for analysis.

2.3.2. **Procedure:** HPTLC analysis was performed on a CAMAG semi automated HPTLC system equipped with an automatic TLC sampler (ATS4); TLC scanner 3 integrated with documentation device Reprostar 3 with win CATS version 1.4.4 planer chromatography manager software. UV cabinet and automatic developing chamber ADC2 with humidity control facility was used for the analysis. 2 ml of substance of each sample added with 2 ml of ethanol. The solution was filtered and 10 μl of filtrate was applied on merck aluminum plate60 F254 Precoated with silica gel of 0.2mm thickness. The samples were loaded in 10 mm bands at 10 mm from the bottom, 15 mm from the sides and with 8 mm space between the two bands. The

plates were developed to a a height of about 8 cm from the base in toluene: ethylacetate (5:2). Plates were developed in software controlled CAMAG automatic developing solvent phase for 30 min at room temperature (25 $\pm$ 20C) and relative humidity was maintained at 45 $\pm$ 1 % with the mobile phase (25 ml) consisting of Toluene : Ethylacetate (5:2) after development , the plates were removed, dried and the spots were visualized under UV light. The mobile phase and chamber conditions resulted in plate was performed in the reflectance /absorbance mode at 254 nm using deuterium lamp in CAMAG HPTLC instrument with following conditions: slit width 6mm $\times$ 0.3 mm, scanning speed 20 mm/s and resolution 100 μ/step. To check the identity of the bands, UV absorption spectrum of each standard was overlaid with the corresponding band in the track (Sethi, 1996; WHO, 1998). The Rf value, area of peak percentage of area were calculated from the calibration graph.

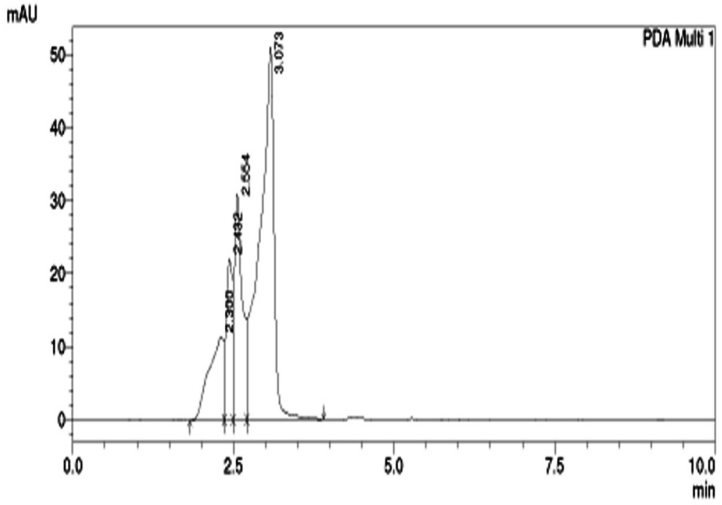
2.4. **HPLC.**

2.4.1. **Preparation of sample solutions:** The methanol extract of each sample was used for HPLC analysis. All extracts were filtered through a Whatman No.1 filter paper. 30ml filtered extract was evaporated by a rotatory vaccum evaporated residues with constant weight were stored prior to analysis in dark at 40C. 200mg of extract was dissolved with 4ml methanol, sonicated at 350C for 15 minutes and filtered through 0.45 μm filter and applied (50μl) on to HPLC column.

2.4.2. **Apparatus and chromatographic conditions:** The HPLC analysis was carried out using Shimadzu, LC-10 AT VP, Consisting of SCL -10 Avp system controller, degassing unit DGU-14 A, low-pressure gradient flow control value FCV-10 ADvp, auto injector SIL-10 ADvp with 500 μl loop, column oven CTO-10 AC, a UV detector SPD-10 Avp with 500μl loop, column oven CTO-10AC, a UV detector SPD-10Avp using a 254 (5μm). The temperature was maintained at 25 $^{\circ}$C with injection volume of 200μl and flow rate of 1ml/min. Active compounds were separated using reverse-phase LiChrosorb C-18 column with the methanol: water mobile phase and detected at 210nm. HPLC conditions for analysis of samples were achieved by varying mobile phase composition 80% methanol in a gradient with 100% methanol. The chromatography system was equilibrated by the mobile phase. When same retention times and peak areas for repetitive injections of standard were observed, separation of sample could then be carried out. Calibration data calculated from peak area and height at different retention time were compared with standard. The peak area of each sample was plotted against the concentration to obtain the calibration graph.

3. RESULTS AND DISCUSSION

3.1. **HPLC Analysis:** The qualitative HPLC fingerprint profile of methanol extract of *Elytria imbricata* was selected at a wavelength of 210nm due to the sharpness of the peaks and proper baseline.



PeakTable

PDA Ch1 220nm 4nm

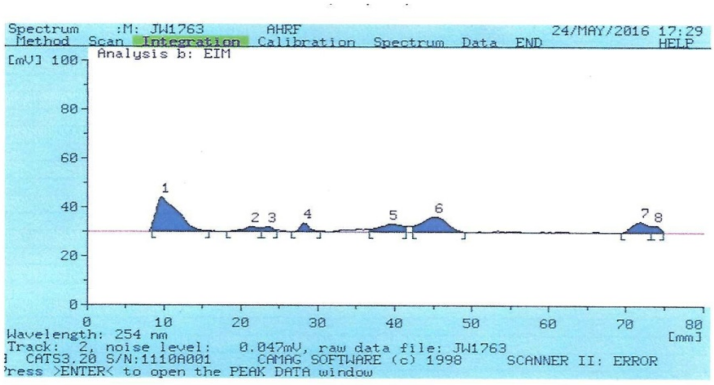
Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.300	183824	11302	13.357	9.803
2	2.432	149644	22009	10.873	19.089
3	2.554	261852	30987	19.026	26.876
4	3.073	780961	50998	56.744	44.232
Total		1376281	115296	100.000	100.000

FIGURE 1. HPLC chromatogram methanol extract of *Elytria imbricata*

The methanol extract prepared by hot extraction was subjected to HPLC for the separation and identification of constituents present in the *Elytria imbricata* four compounds were separated at different retention times of 2.300min, 2.432min, 2.664min and 3.073min respectively. The profile displayed three prominent peaks at the retention times of 2.432min, 2.664min and 3.073min followed by one moderate peak was also observed at the retention times of 2.300 min as shown in **Figure 1**.

3.2. **HPTLC fingerprint:** The qualitative HPTLC fingerprint profile of methanol extracts of *Blepharis repens* were obtained and the chromatogram were observed under 200-400nm. The densitogram data was analyzed.

8 peaks were eluted in the methanol extract of *Elytria imbricata* the maximum height 14.3AU with Rf value 0.10 was observed in 1^{st} peak and maximum height and a maximum percentage



S/NO	RF	HEIGHT	AREA	LAMDA MAX(nm)
1	0.10	14.3	458.8	326
2	0.21	2.0	49.3	362
3	0.23	2.1	34.4	362
4	0.28	3.6	52.2	362
5	0.39	3.3	117.8	221
6	0.45	6.5	249.0	220
7	0.72	4.5	105.0	338
8	0.74	3.1	40.5	283

FIGURE 2. HPTLC densitogram methanol extract of *Elytria imbricata*

of area (458.8%). The 2^{nd} peak had the minimum height 2.0 AU with Rf value (0.21) and a minimum percentage of area (49.3%) as shown in **Figure 2**.

4. CONCLUSION

In conclusion, the results obtained that HPLC and HPTLC finger print can be used as a for the plant. The methanol extract of *Elytria imbricata* were used for HPTLC and quantification of active compounds by HPLC method. HPTLC analysis had different number of compounds and in HPLC analysis the active compounds were quantified which varied from methanol extract of the sample.

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