

Research Article

Thin Layer Chromatographic Method for Quantification of Swertiamarin in Whole Plant Powder of *Enicostema littorale*

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ABSTRACT

A sensitive simple and accurate HPTLC method has been developed for the quantification of swertiamarin in dry whole plant powder of *Enicostema littorale* (Lam). Chromatographic analysis was performed using chloroform alcoholic and aqueous extract on silica gel 60 F₂₅₄ HPTLC plate, using a solvent system, comprising of Ethyl acetate methanol (8:2) as mobile phase gives good separation of swertiamarin at R_f 0.56. Detection and swertiamarin was done by densitometric scanning at $\lambda=254$ nm. The concentration of swertiamarin in the alcoholic extract of dried powder of *Enicostema littorale* by the proposed HPTLC method. The identity of swertiamarin band in the sample extract was confirmed by overlapping UV absorption spectrum of the sample with that from the reference standard swertiamarin using the CAMAG TLC scanner 3. The proposed HPTLC method provided a good resolution of swertiamarin from other constituents present in the extracts could be used for a routine quantity control analysis and quantification of swertiamarin in the whole plant extracts of *Enicostema littorale*.

Key words: *Enicostema littorale*, Chota chirayita, HPTLC, TLC.

INTRODUCTION

Enicostema littorale belongs to family Gentianaceae. Locally it called as 'Vellaragu'. Gentianaceae is an erect annual herb growing to height of about a metre, and indigenous to the mountainous Districts of Northern India. *Enicostema littorale* is an annual or perennial herb. Stems often winged, rounded or angular leaves, sessile, often narrow. Inflorescence axillary dense clusters or cyme. A glabrous procumbent perennial herb up to 50 cm in height. From a thick root stock, stem & branches sub quadrangular; leaves simple opposite, sessile linear oblong or elliptic lanceolate glabrous 3-nerved marginal nerves often obscure flowers white tubular in whorled axillary clusters. Calyx narrow campanulate divided down halfway to 2/3, thin with white thinner margins; corolla is small, white tubular and funnel shaped. Stamens inserted in corolla tube, with appendices at filament bases; filament equal length anthers erect after anthers with sterile apex. Ovary without nectary disk stigmas capitate slightly bilobed. Fruit ellipsoid capsule narrow at the base & rounded not winged.

Enicostema littorale Blume, Gentianaceae) is a perennial herb found throughout India. And common in coastal areas. The plant is used in Folk medicine to treat diabetes mellitus, rheumatism abdominal ulcers. Hernia, swelling itching hypoglycemic,³⁻⁵⁾ and anticancer⁶⁾ activities have been reported. These reported activities and many of the ethnomedical Uses of the plant are related to its antioxidant activity. Swertiamarin, alkaloids, steroids, triterpenoids, saponins flavonoids, xanthone.⁴⁾ Many such compounds has protective effects due to their antioxidant properties.⁷⁾

HPTLC method has enlarged as an efficient tool for phytochemical evaluation of herbal drugs. HPTLC method has been devised in the present research work which may be used as an alternative method for quantification of swertiamarin from *Enicostema littorale* whole plant powder and also for the standardization of *Enicostema littorale* using swertiamarin as marker. The proposed HPTLC method is simple, precise and sensitive.

MATERIALS AND METHODS

HPTLC Fingerprinting For Methanolic Extract Of *Enicostemma Littorale*: As a result of column chromatography, the plant extract disclosed two significant fraction one in Ethyl acetate and other in methanol fraction. These fractions were subjected to TLC, FT-IR, GC-MS assays.

HPTLC Profile of Methanol Fraction, Scanning at 254 nm : High performance Thin Layer Chromatography (HPTLC) was carried out for the methanol fraction of *Enicostemma littorale*. HPTLC Profile of various solvents of Petroleum ether ethyl acetate, Methanol Fraction on "Silica gel "G" Fig. Using Ethylacetate: Methanol (8:2) as mobile phase revealed 5 spots in UV-366 nm. 5 μ l concentration applied on precoated silica gel 60 F 254 HPTLC Fig. ethyl Acetate methanol (8:2) was used as a mobile phase and scanned at 254 nm. Methanol fraction of *Eniocstemma littorale* revealed 5 peaks. The R_f values and the peak area percentage were observed and given in (table 10 & 11) and illustrated as chromatograms. High performance Thin Layer Chromatography (HPTLC) was carried out for methanol fraction of *Enicostemma littorale*. 15 μ l concentration applied on a precoated silica gel 60 F 254 nm. Petroleum

PETROLEUM ETHER EXTRACT

Fig.1 Petroleum Ether



ETHYLACETATE EXTRACT



Fig.2 Ethyl acetate



Ether: Ethyl Acetate (8:2) was used as mobile phase and scanned at 366 nm and it depicted 8 peaks The R_f values and the peak area percentage were observed as per the above table.

Test solution: Defating 5g of the finely powdered plant material with 25ml of solvent ether and refluxing with 25ml of methanol for 25minutes followed by filtering and removing the solvent under reduced pressure. Add in a test solution 25mg of the extract in 10ml of methanol was resuspended.

Solvent System : Ethyl acetate: Methanol (8: 2)

Procedure: 5 μ l and 15 μ l of the test solution when applied on precoated silica gel 60F₂₅₄. (E.Merck) of uniform thickness of 0.2mm, and developed in the solvent system to a distance of 8cm.

Visualization: It was observed under UV light at 254 nm

and 366 nm, and then scanned at 243nm. For the R_f values and colours of the resolved bands. The output was sprayed with anisaldehyde –sulphuric acid reagent and heated at 110°C until the coloured bands appeared.

Evaluation: According to the reference, a band at R_f 0.56 could be Swertiamarin and it was visible in test solution as well (Track 2).The three dimensional super imposable spectra of Methanol fraction of *Enicostemma littorale* at 254 nm.HPTLC Profile of Methanol Fraction of *Enicostemma littorale*-Scanning at 254 nm.

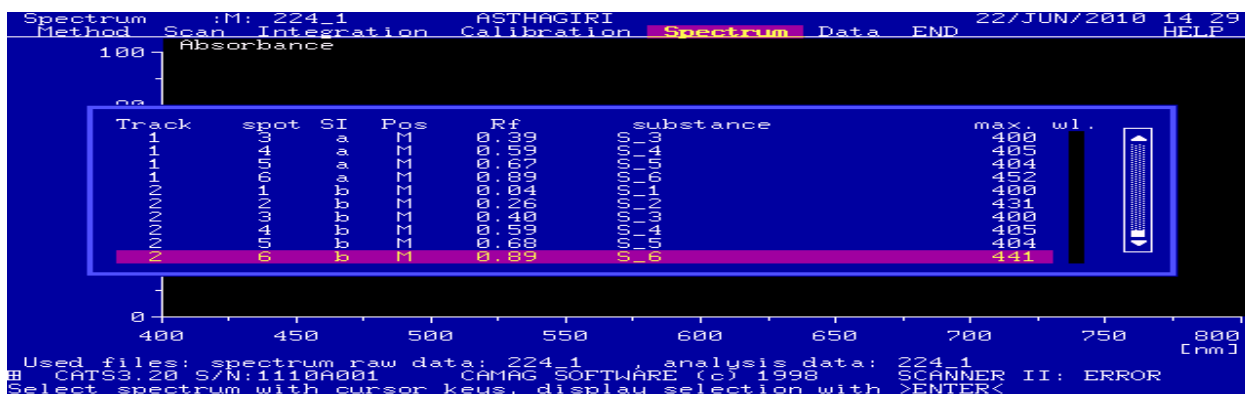
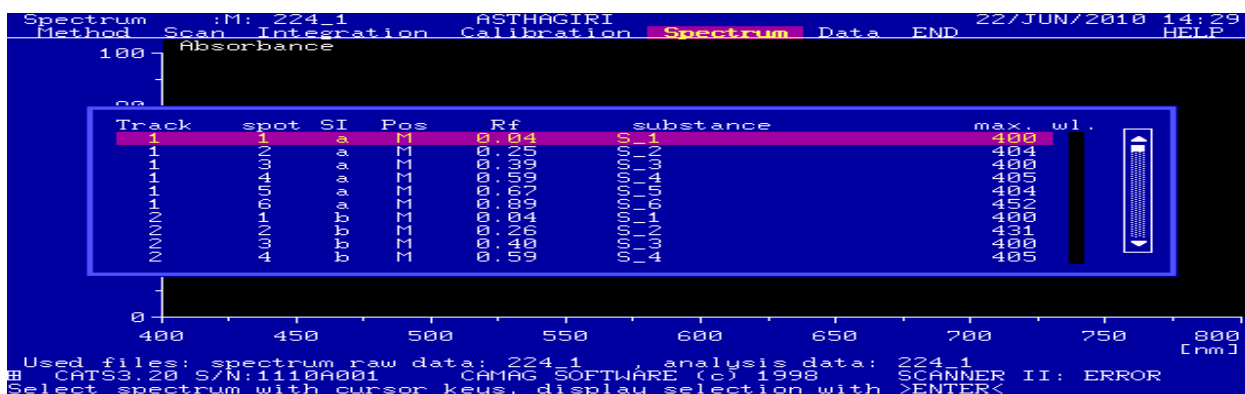
Super impossible Spectra of Methanol Fraction

Mobile Phase : Ethyl Acetate: methanol (8:2)

Application Volume : 5 μ l, 15 μ l

Scanning : 254 nm

RESULT & DISCUSSION



The method as described in the present study, utilize silica gel 60F₂₅₄ TLC plate as stationary phase and **Ethyl Acetate: methanol (8:2)** as mobile phase gives good separation of swertiamarin at R_f =0.56 , from the other components present in whole plant powder of *Enicostema littorale* Lam. The TLC plate was visualized under UV light at 254 nm and the HPTLC photographed chromplate

is showed. The identity of the swertiamarin band in the sample extract was confirmed by overlaying the UV absorption spectrum of the sample with that from the reference standard swertiamarin, using the Camag TLC scanner 3.

CONCLUSION

Table 1. HPTLC Finger Print of Methanol Fraction (5 µl)

S.NO	R _f	% Area
1.	-0.05	15.59
2.	0.05	2.82
3.	0.10	6.33
4.	0.37	70.63
5.	0.92	4.63

Fig.3 Methanolic extract of HPTLC

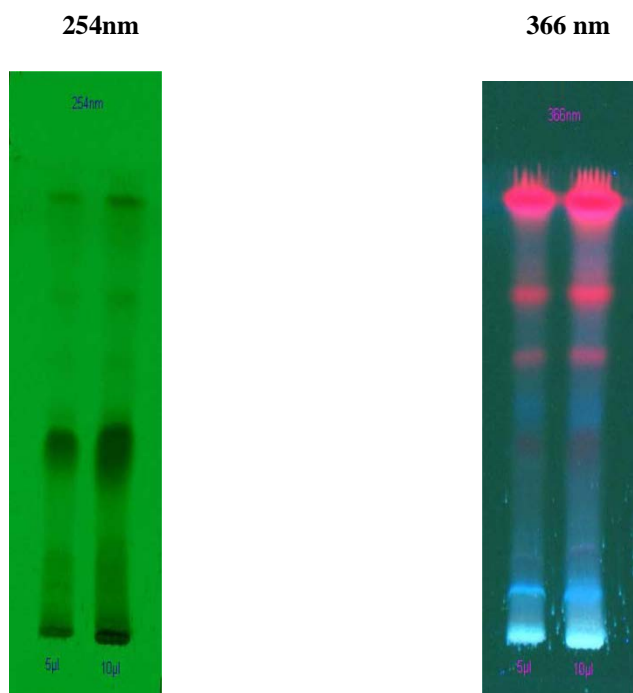


Table 2: HPTLC Finger Print of Methanol Fraction(15µl)

S.NO	R _f	% Area
1.	-0.05	2.00
2.	-0.03	0.65
3.	0.09	4.49
4.	0.15	1.12
5.	0.39	75.48
6.	0.56	1.05
7.	0.70	3.54
8.	0.84	3.36
9.	0.92	8.33

The proposed HPTLC method developed a good resolution of swertiamarin from other constituents present in the extracts of *Enicostema littorale* (Lam). The method

can be used for a routine quality control analysis and quantitative determination of swertiamarin in the whole plant powder extract of *Enicostema littorale*.

Fig 4.HPTLC Finger Print of Methanol (5µl)

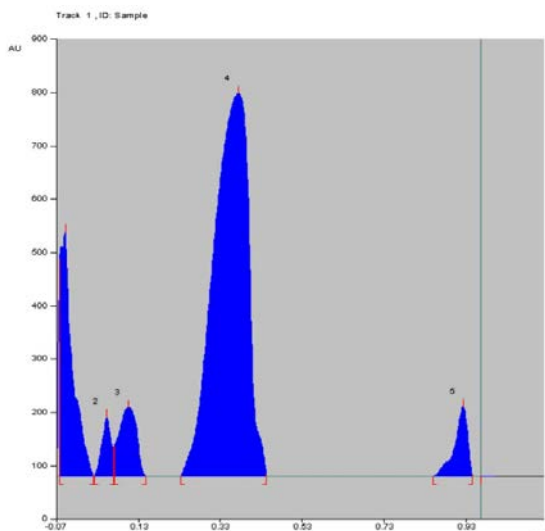


Fig.5 HPTLC Finger Print of Methanol (15µl)

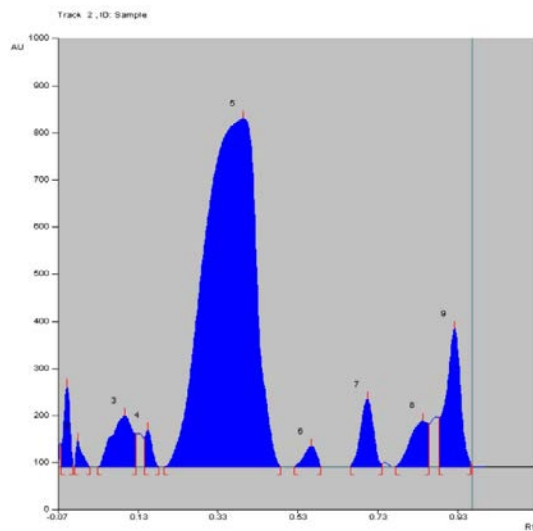
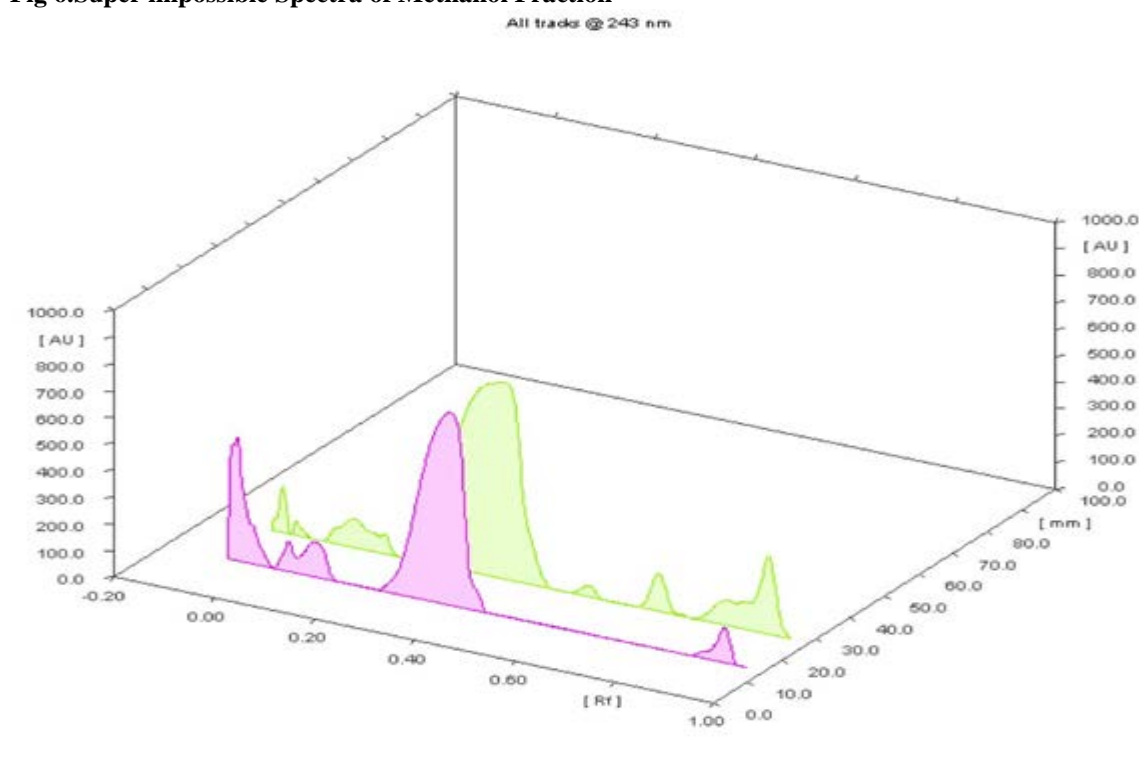


Fig 6.Super impossible Spectra of Methanol Fraction



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