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HPTLC QUANTIFICATION OF n-OCTACOSANOL IN *TINOSPORA CORDIFOLIA* MIERS STEMS OBTAINED FROM DIFFERENT GEOGRAPHICAL REGIONS

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ABSTRACT

n-octacosanol is an normolipidemic aliphatic primary alcohol obtained from the plant *Tinospora cordifolia* Miers. In the present study, the plant stems were collected from five accessions namely Anniyalam, Chennai, Hassan, Vishakhapatnam and Bangalore between June and August. Later a simple HPTLC method has been established for determination of n-octacosanol in methanolic extracts of all these geographical sources using Toluene: Ethyl acetate: Formic acid (4.5:4.5:1 v/v) as mobile phase. Detection and quantification were performed by densitometric scanning at λ = 555 nm. The results of HPTLC estimation showed variation in n-octacosanol content in all the accessions. The extract from Anniyalam accession showed significantly higher amount of n-octacosanol (6.54%) and that of Vishakhapatnam showed the least (2.28%). Thus, the present work is helpful in selecting genotype of *Tinospora cordifolia* yielding highest percentage of n-octacosanol.

INTRODUCTION: *Tinospora cordifolia* Miers (Menispermaceae) is widely used in the Indian system of medicine to improve immune system and the body resistance against infections [1]. It is reported to possess antispasmodic, anti-inflammatory, antiallergic and anti-diabetic properties.

It is generally prescribed in general debility, diabetes, fever, jaundice, skin diseases, rheumatism, urinary diseases, dyspepsia, gout, gonorrhoea and leucorrhoea [2].

It also possesses antineoplastic activity [3]. n-octacosanol isolated from *Tinospora* is useful in improving endurance and increasing oxygen utilization [4]. Limited studies suggest that n-octacosanol works to reduce blood cholesterol [5] and helps to treat the patients suffering from coronary heart diseases [6], Parkinson's disease [7, 8, 9].

The variations of chemical constituents are observed in plants belonging to the same species grown in different environmental conditions. These variations may occur due to climatic conditions like altitude, temperature, type of soil etc.

The plants collected from different geographical sources have shown similar morphological characters but difference in content of secondary cell constituents.

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Hence, in the present study, an attempt was made to select an appropriate genotype that produces maximum amount of n-octcosanol. This leads to the production of plants with better market value. Hence, it is very essential to select the correct accession for the particular usage and for further cultivation.

MATERIALS AND METHODS:

Plant material: Plant materials were collected from Anniyalam, Chennai, Hassan, Vishakhapatnam and Bangalore between June and August and were taxonomically identified and authenticated by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. The Voucher specimen (TC-001, TC-002, TC-003, TC-004 and TC-005) were deposited in herbarium of Natural Remedies Private Limited, Bangalore for future reference.

Preparation of Plant Extracts: The stems were washed, dried under shade and around 50g from each accession were powdered to pass through 40-mesh sieve. The drug was extracted with methanol (100ml x 3) over steam water bath for 3-hours. After cooling, the solvent was removed on a rotary vacuum evaporator. The extracts were stored in desiccators and protected from the light.

Reagents and Chemicals: HPLC grade Methanol, Toluene and ethyl acetate (Qualigens) while formic acid is of analytical grade.

HPTLC Quantification in Test Samples: A Camag HPTLC system equipped with Shimadzu CS9301 PC densitometer, TLC scanner 3 and integrated software WINCATS version 1.4.1 was used for the analysis.

Reference Standard Preparation: 2.00 mg of standard n-octacosanol as reference compound procured from Natural Remedies Pvt. Ltd. Bangalore was accurately weighed in an electronic balance (Afcoset) and dissolved in 1ml chloroform and 3ml methanol, sonicated for 5-10 min. This solution was used as reference solution for HPTLC analysis.

Test Solution Preparation: 25 mg of all the extracts were weighed separately, dissolved in methanol and sonicated. Filtered solutions were then used as test solutions for HPTLC analysis.

Sample Application: 20µl of test solution and standard solution (0.5mg in 4ml solvent) was loaded as 6mm band length in 20 cm × 10 cm HPTLC plates coated with silica gel 60F254 (E.Merck Germany) of 200 µm layer thickness using Camag Linomat (V) sample applicator equipped with a 100 µl microsyringe and an automatic TLC sampler (ATS4) under the flow of nitrogen gas.

Spot Development: The samples applicated plate was kept in TLC twin trough developing chamber (after saturating with solvent vapor) having Toluene: Ethyl acetate: Formic acid (4.5: 4.5 :1) as mobile phase (n-octacosanol) and the plate was developed in the respective mobile phase up to 80mm.

Derivatization: The developed plate was sprayed with ANS spray reagent. Before scanning, plates were heated for 5 min in oven at 105°C in hot air oven.

Photo-Documentation: The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the image in UV at 254nm and 366nm.

Scanning: Densitometric scanning of the plate was performed at λ_{max} = 555 nm using a Camag Scanner III in absorbance mode with tungsten lamp in conjunction with WINCATS software for quantification. Slit dimension was 10.00 mm X 0.90 mm.

Detection: Blue coloured band at 555nm was present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of n-octacosanol in all the samples. The Peak table, Peak display and Peak densitogram were noted. The amount of n-octacosanol in test samples were determined according to the formula.

Formula:

%Content of n-octacosanol =

$$\frac{\text{Standard area} \times \text{Sample weight} \times \text{Standard weight} \times \%\ \text{Purity of the standard}}{\text{Sample area} \quad \text{Sample dilution} \quad \text{Standard dilution}}$$

RESULTS AND DISCUSSION: Under the chromatographic conditions described above the retention time for n-octacosanol was found to be 1.06. The chromatogram of standard n-octacosanol and that of test samples are shown in the **figure 1-6**

respectively. The analysis indicated the presence of optimum amount of n-octacosanol in Anniyalam accession (6.54%) and Chennai (5.33%). The content of n-octacosanol was observed to be 4.78%, 4.68% and 2.28% in Hassan, Bangalore and Vishakhapatnam methanolic extracts respectively (**Table 1**).

TABLE 1: PERCENTAGE CONTENT OF N-OCTACOSANOL PRESENT IN *TINOSPORA CORDIFOLIA* STEMS FROM DIFFERENT GEOGRAPHICAL ORIGIN

Fig. No.	Samples (MeOH extracts)	Injection Volume	Retention time (min)	Area	% Content of n-otacosanol
1	n-octacosanol	20µl	1.06	6819.0	Ref. std.
2	**Anniyalam**	20µl	1.06	4492.6	**6.54%**
3	Bangalore	20µl	1.05	3054.7	4.68%
4	Chennai	20µl	1.06	3529.7	5.33%
5	Hassan	20µl	1.05	3066.9	4.78%
6	Vishakhapatnam	20µl	1.06	1501.4	2.28%

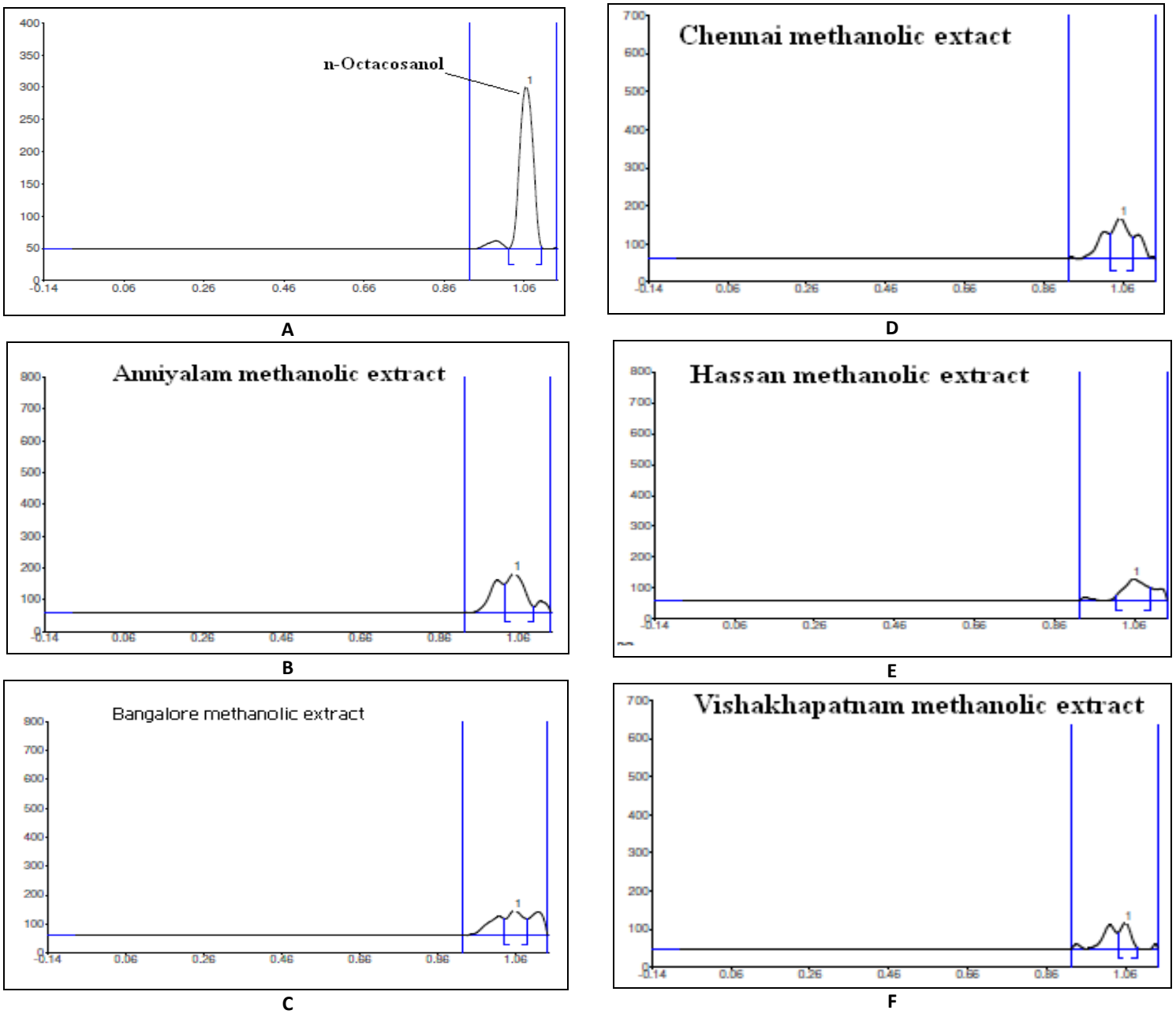

FIG. 1: HPTLC CHROMATOGRAMS OF (A)n-OCTACOSANOL, METHANOLIC EXTRACTS FROM THE STEMS OF *TINOSPORA CORDIFOLIA* FROM (B) ANNIYALAM (C) BANGALORE (D) CHENNAI (E) HASSAN AND (F) VISHAKHAPATNAM

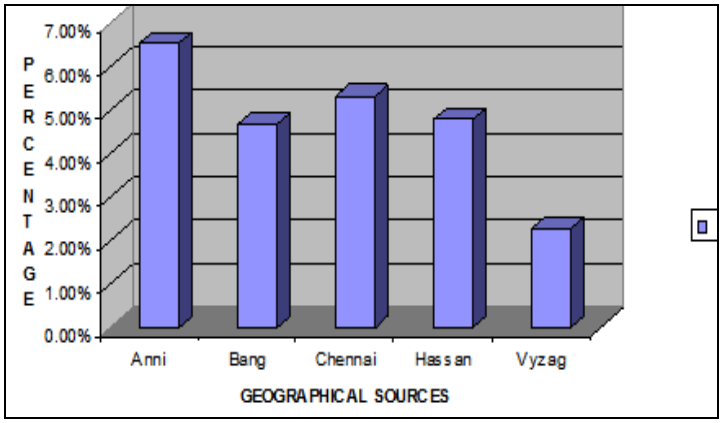

FIGURE 2: BAR CHART REPRESENTING PERCENTAGE OF n-OCTACOSANOL PRESENT IN METHANOLIC EXTRACT OF *TINOSPORA CORDIFOLIA* COLLECTED FROM DIFFERENT GEOGRAPHICAL REGIONS

CONCLUSION: n-octacosanol is an important high molecular weight primary fatty alcoholic compound in *Tinsopora cordifolia* Miers. A TLC densitometric HPTLC method for the quantification of this compound in methanolic extracts of accessions of *Tinospora* from various geographical sources has been established. The highest percentage of n-octacosanol was found to be present in Anniyalam accession.

The method was found to be simple and sensitive. As n-octacosanol has been found to be present in all the sources, it can be used in routine quality control of herbal materials as well as formulations containing n-octacosanol and the present work is helpful to select an appropriate genotype of *Tinospora cordifolia* producing maximum amount of n-octcosanol, which is novel of its kind.

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