Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2014; 3(4): 171-175
Received: 09-09-2014
Accepted: 14-10-2014

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Comparative HPTLC fingerprint profile of triterpenes in the methanolic extracts of *Ocimum basilicum* L. and *Mentha arvensis* L. (Lamiaceae)

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Abstract
A comparative account of the Triterpenes present in the methanolic extract of *Ocimum basilicum* L. and *Mentha arvensis* L. were done using HPTLC. Plant materials were extracted with methanol in a soxhlet extractor. CAMAG HPTLC system equipped with Linomat 5 applicator, TLC scanner 3 and WIN CATS software were used. The methanol extract of the plants were fingerprinted in Toluene:Chloroform:Ethanol (4:4:1) solvent system and scanned densitometrically under UV using anisaldehyde sulphuric acid as reagent. This study can be taken as a tool for chemotaxonomic identification, useful in differentiating the species from the adulterant.

Keywords: *Ocimum basilicum* L., *Mentha arvensis* L., HPTLC finger printing, Triterpenes.

1. Introduction
Many medicinal plant extracts in their aqueous or alcoholic forms are used in manufacturing Ayurvedic and herbal formulations. If the phytochemical profile of the plant or its part is known an appropriate kind of extract can always be used by selection for a particular purpose. Chemical fingerprinting is an effective tool in authentication and quality control of herbs. Chromatography can readily ascertain the presence of the essential chemical constituents of a medicinal plant and detection of their presence in a preparation [1, 2].
Consumption of fruit and vegetables has been associated with a lower incidence of cancer and other diseases. Diets, especially along the Mediterranean coast, are correlated with healthiness [3]. Mediterranean spices and fruits contain, pentacyclic triterpenes that are regularly isolated as active substances from these plants. They can be found in spices of the *Lamiaceae* family as well as within olive leaves and fruit. Virgin olive oil contains up to 197 mg/kg triterpenes, indicating the importance of these substances as nutraceuticals [4-6]. Simple and conjugated triterpenes have a wide range of applications in the food, health, and industrial biotechnology sectors.
Ocimum basilicum L. also called 'Sweet Basil' has been used for thousands of years as a medicinal herb and a spice. It acts principally on the digestive and nervous systems [7]. According to Ayurveda, *Ocimum basilicum* L. used as a stomachic, anthelmintic, antipyretic, improves the taste, indigestion, useful in diseases of the heart and blood diseases. Some studies have reported its antioxidant, radical scavenging, antiinflammatory, antiulcer and cardiac stimulant activity [8, 9].
Mentha arvensis L. is traditionally used to treat flatulence, digestion problems, gall bladder problems and coughs. The aromatic leaves are used widely for flavouring foods and beverages. It is used as a contraceptive [10], carminative, antispasmodic, anti-peptic ulcer agent, and has been given to treat indigestion, skin diseases, coughs and colds in folk medicine [11]. Therefore the selected medicinal plants of the same family proved highly significant in terms of presence of therapeutically active secondary metabolites.
HPTLC is being used for fingerprint profiling of medicinal plant extracts since long [12, 13]. The HPTLC fingerprint profile has been proved to be an effective tool in differentiating closely related species and detecting adulteration and substitution in raw drugs of Indian systems of medicine [14].

This study was conducted to derive a fingerprint profile of Triterpenes in the methanolic extracts of *Ocimum basilicum* L. and *Mentha arvensis* L. by HPTLC. In the present investigation, considering the highly significant therapeutic role of phytochemicals, an effort was made to find comparative HPTLC fingerprinting of methanolic extracts of two different genus of the same family.

2. Materials and methods

Aerial parts of the plants used in the present study were collected, washed and air dried under shade (two weeks). The dried plant parts, finely powdered using electric grinder, sieved (mesh size 500 µ) and subjected for the extraction. Powdered samples were extracted with 200 ml of methanol for 8-12 h using the Soxhlet apparatus. Quantitative phytochemical characterization was done using High Performance Thin Layer Chromatography [15, 16]. For this, CAMAG HPTLC system equipped with Linomat 5 applicator, TLC scanner 3 and WIN CATS software were used. High-Performance Thin-Layer Chromatography was performed on silica gel 60F254 (10 cm× 10 cm; Merck). Methanol extract of the selected plants (10 mg/ml) and collected fractions residue (1 mg/ml) was subjected to HPTLC (CAMAG, Switzerland) analysis. Extract and each fraction were spotted on a silica gel 60F254 (Merck, Germany) TLC plate. The plate was air dried and then developed by using the solvent system Toluene: Chloroform: Ethanol (4:4:1) as mobile phase in a CAMAG-twin-trough glass chamber (20x10x4) previously saturated with mobile phase vapor for 20 min. After developing the plate, it was dried at 105 ºC for 15 min and then it was scanned using Scanner 3 (CAMAG, Switzerland) at 366 nm using WinCATS software. Chromatograms were evaluated before and after spraying with Anisaldehyde sulphuric acid reagent.

3. Results and discussions

The methanol extracts of *Ocimum basilicum* L. and *Mentha arvensis* L. were fingerprinted by HPTLC. The phyto-chemical constituents in a plant material from a characteristic fingerprint, representing the quantity of active constituents. Moreover, this helps to standardize the mixtures like herbal drug formulations and market samples [17]. However the HPTLC technique is having precision over TLC method and thus an effort was made in the present study to develop comparative HPTLC profiles of Triterpenes in the methanolic extracts of two taxa of the same family. The HPTLC densitometric scanning results observed in the present study are stated below.

Ocimum basilicum L. methanol extract showed excellent bands about 12 in number, in the solvent system Toluene: Chloroform: Ethanol (4:4:1). (Figure-1 a & b) (Table 1). When viewed under UV at 254 nm, 366 nm and after derivatization with Anisaldehyde sulphuric acid reagent, good separation of constituents with different Rf values were observed (Figure-2 a & b). Methanol extract of *Mentha arvensis* L. also showed 11 bands in the same solvent system with different Rf values. Rf values of Triterpenes in the total methanolic extract of *Ocimum basilicum* L. were compared with that of *Mentha arvensis* L. (Table 1). Both plants showed same triterpene compounds in the Rf values 0.13, 0.20, 0.35, 0.43 and 0.78. Bandwidth and peak area was the maximum in the band with Rf 0.06 for *Ocimum basilicum* L. and minimum with Rf 0.86 (Figure 3). *Mentha arvensis* L. showed the maximum peak area in the Rf 0.08 and least width with Rf 0.78. (Figure 4). This fingerprint profile of Triterpenes in these plants can be used for the identification and quality control of the extracts. Both plants share common bands which indicate their chemical similarity as a single family. In addition to the common bands they also showed characteristic bands which may indicate their typical genera character. The extracts of the aforementioned medicinal plants justify further studies to isolate and characterize the active constituents. It is important to develop a better understanding of their mode of therapeutic action for further applications.

Table 1: Comparative chart of Peak, Rf, Maximum height and Area of methanolic extract of *Ocimum basilicum* L. and *Mentha arvensis* L.

***Ocimum basilicum* L.**				***Mentha arvensis* L.**			
Peak	**Rf**	**Max. Height**	**Area**	**Peak**	**Rf**	**Max. Height**	**Area**
1	0.06	150.2	5207.5	1	0.08	172.0	4918.1
2	0.13	92.8	2702.5	2	0.13	94.7	4536.5
3	0.20	24.7	493.2	3	0.20	76.1	1445.8
4	0.27	25.0	670.7	4	0.24	73.1	2652.9
5	0.35	139.9	3630.5	5	0.32	83.7	2739.1
6	0.43	105.6	3105.8	6	0.35	136.4	3734.7
7	0.49	124.6	2661.1	7	0.43	70.8	1880.6
8	0.55	42.7	1129.5	8	0.47	38.0	773.5
9	0.69	28.0	783.9	9	0.53	17.5	559.0
10	0.72	37.0	784.2	10	0.66	12.5	381.3
11	0.78	84.2	2326.2	11	0.78	15.6	377.6
12	0.86	27.7	248.0	-	-	-	-

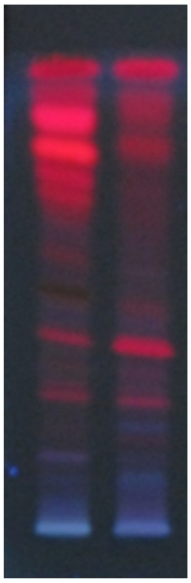

Fig 1a: Developed HPTLC plate prior to derivatization under UV 366nm

Fig 1b: Developed HPTLC plate prior to derivatization under UV 254 nm

Track 1: Methanolic extract of *Ocimum basilicum* L.
Track 2: Methanolic extract of *Mentha arvensis* L.
Solvent system- Toluene: Chloroform: Ethanol (4:4:1).

Fig 2a: After derivatization under visible light

Fig 2b: UV 366 nm after derivatization

Track 1: Methanolic extract of *Ocimum basilicum* L.
Track 2: Methanolic extract of *Mentha arvensis* L
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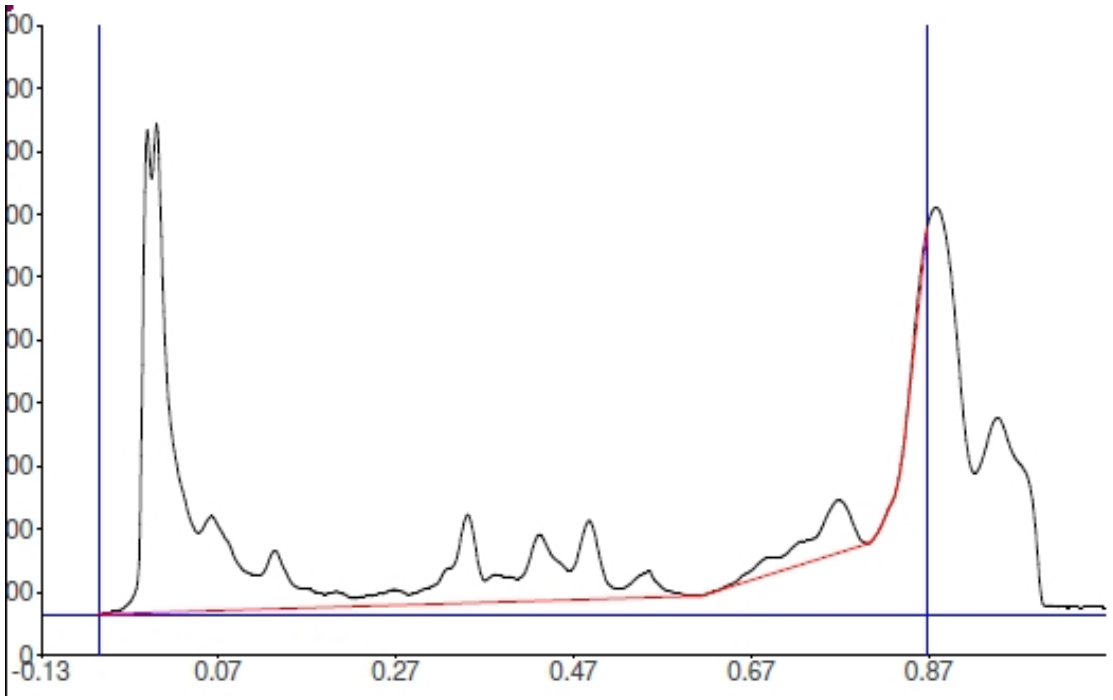

Fig 3: HPTLC of methanolic extract of *Ocimum basilicum* L.

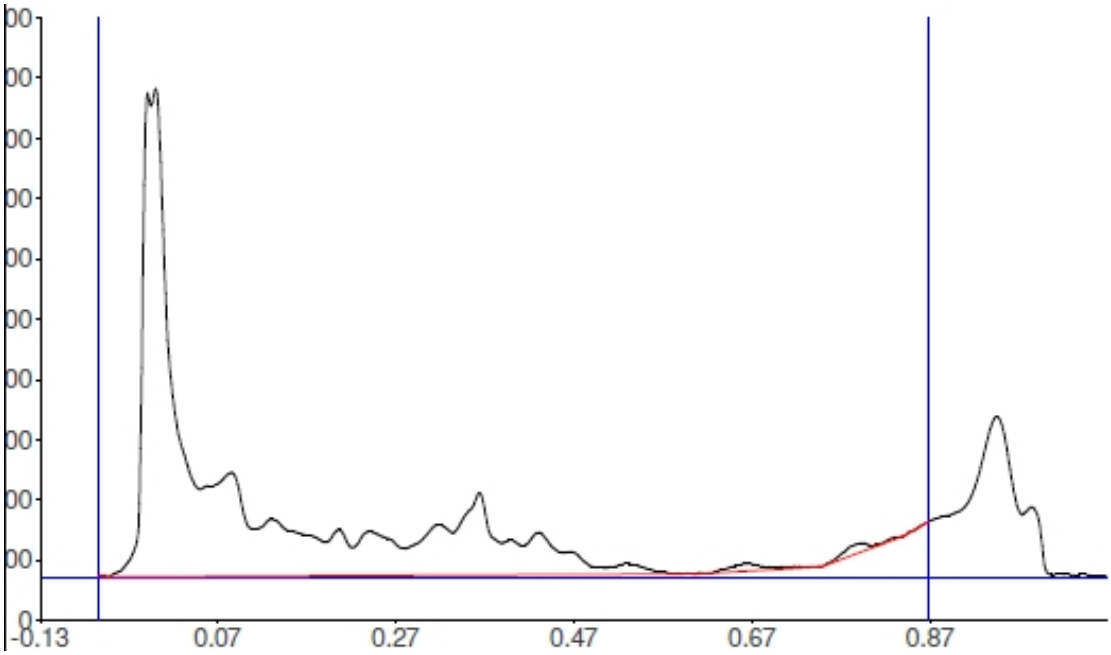

Fig 4: HPTLC of methanolic extract of *Mentha arvensis* L.

4. Acknowledgements
The authors wish to thank the support of staff at the New Udaya Pharmacy and Ayurvedic Laboratories, Cochin.

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