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HPTLC fingerprint profile of *Coriandrum sativum* Linn.

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ABSTRACT

To develop the finger print of medicinally and economically important plant of *Coriandrum sativum* Linn[Family: Apiceae]. Different extracts of fresh leaves and dried fruits of C.sativum were developed in the mobile phase of Toluene: ethyl acetate: formic acid::45:5:1 using standard procedure[10µl] and scanned under UV at 254 nm and 366 nm. The HPTLC fingerprinting of he different extract have shown several peaks with different Rf values. Petroleum ether extract of fresh leaves[maceration] and dried fruit [Soxhlation and maceration] has shown 5, 9 and 10 compounds; benzene extract showed 10,9 and 7 compounds; chloroform extract showed 8,8 and 9 compounds; methanol extracts showed 7,6 and 9 compounds; ethanol extracts showed 7,6,and 14 compounds and water extracts sowed 6,9 and 7 compounds respectively. It can be concluded that HPTLC fingerprint analysis of leave and fruit extract of *Coriandrum sativum* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker.
Keywords: *Coriandrum sativum*; HPTLC, Fingerprinting, Medicinal plant, Soxhlation, Maceration.

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INTRODUCTION

The human being exploited to alleviate his sufferings from injuries of deceases utilizing plant growing around him. The plant kingdom still hold many species of plant containing substance of medicinal value which have yet to be discovered and he large number of plants are constantly being screened for their possible pharmacological value in addition to already exploited plants [1]. As the results of modern isolation technique and pharmacological screening procedure, new plant drugs usually find their way into modern medicines. Now a days maximum world population depends on herbal medicines and plants remain a major source of medicinal compounds. Synthetic drugs causes side effects as a result, people are more favorable to usenatural compounds obtained from plants [2]. It has been estimated that 56% of the lead compounds for medicines in the British National Formulary are natural products [3]. Phytochemical analysis of plants which are used in Traditional system of Medicine has yielded a number of compounds with various pharmacological activities. Standardization of crude plant drugs/ herbal medicines is the need of the day. Several pharmacoepoeias containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constitutnets in the plant material may be useful for proper standardization of herbals and its formulations [4]. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [5]. Chromatographic fingerprinting techniques are most significant methods which can be used for routine herbal drug analysis and for quality assurance. Currently HPTLC is often used as an alternative to HPLC for the quantification of plant products because of its simplicity, accuracy, cost –effectiveness and rapidity [6]. HPTLC offers better resolution and estimation of active constitutents is done with reasonable accuracy in a shorter time [7]. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to Indian Traditional medicines. The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs. HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug [8]. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduce time and cost of analysis. In addition. It minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environmental pollution.HPTLC also facilitates repeated detection of chromatogram with same or different parameter [9].

Coriander[*Coriandrum sativum* Linn.] an annual of the Apiaceae family is one of valuable medicinal and seasoning plant. This species comes from the Mediterranean region and it is grown all over the world. The coriander fruit and essential oil isolated from it are used for medicinal purpose. It is used to treat menstrual disorder, secondary infertility, ovaritis and cervicitis. It is used to treat female diseases such as menoxenia, ovulation type dysfunctional uterine bleeding [10]. It is aphrodisiac to enhance sexual function and reproductive capacity. It is used for treating leucorrhea, spermatorrhea.Coriander fruit possess stimulant and carminative properties[11]. Its oil is bactericidal and larvacidal [12]. It is hypoglycemic and anti-inflammatory [13]. The fruits are used as astringent, anthelmintic, emollient, stomachic, antibilious, digestive, appetizer, constipating, diuretic, antipyretic, refrigerant, tonic, expectorant, anodyne, antidiabetic and dyspepsia [14].

Inspite of its abundant uses, the chromatographic finger print profile of the *Coriandrum sativum* have not been reported. The main objective of this study was to evaluate and optimize the HPTLC fingerprint method in standardization of *Coriandrum sativum*.

MATERIAL AND METHODS

Plant material

The *Coriandrum sativum* fruits and fresh leaves were collected from local market in Bangalore, Karnataka, India and it was identified and authenticated by Botanist, Natural Remedies Pvt Ltd., Bangalore. A voucher specimen was deposited in The Oxford College of Pharmacy, Bangalore. The fruits were dried in shade and powdered coarsely, passed through sieve no. 40 and stored in air tight container for further use. Whereas fresh leaves are washed with water free from extraneous material and cut in small pieces.

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Preparation of fruit extract by soxhlet and maceration technique

Coarsely powdered fruits of *C. sativum* 250 g, each were subjected to extraction in soxhlet extractor and kept for maceration with solvents of increasing polarity from petroleum ether, benzene, chloroform, methanol and ethanol [1500 ml] respectively. Each time, the marc was dried before proceeding to the next solvent. 250 g fruits were extracted separately with distilled water on water bath and kept for maceration. All the six extracts were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield were found to be10.35; 2.36; 1.10; 7.68;0.29; 3.26 and4.35;3.97;3.58;2.54;0.85;5.78 % w/w respectively for soxhlation and maceration technique with reference to the air dried plant material.

Preparation of leaves extract by maceration technique

Coarsely cut fresh leaves of *C.sativum* 250 g, each were kept for maceration for 24 hrs with solvents of increasing polarity from petroleum ether, benzene, chloroform, methanol and ethanol [1500 ml] respectively. Each time, the marc was dried before proceeding to the next solvent. 250 g fresh cut leaves were kept for maceration. All the six extracts were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield were found to be 0.1;.16;.19;1.02;0.49 and 0.84 % w/w respectively for maceration technique. A stock solution was prepared at a concentration of 25 mg/ml and was used for HPTLC.

Instrumentation

A Camag HPTLC system equipped with a sample applicator Linomat V, Twin trough plate development chamber, TLC Scanner with WinCATS software were used.

Sample preparation

A stock solution of different extracts were prepared at a concentration of 25 mg/ml in chromatographic grade methanol which was used for sample application on pre-coated silica gel 60F254 aluminum sheets[E.Merck Ltd, Germany]

Developing solvent system

A number of solvent systems were tried but the satisfactory resolution was obtained in the solvent system Toluene: ethyl acetate: formic acid::45:5:1.

Chromatographic conditions

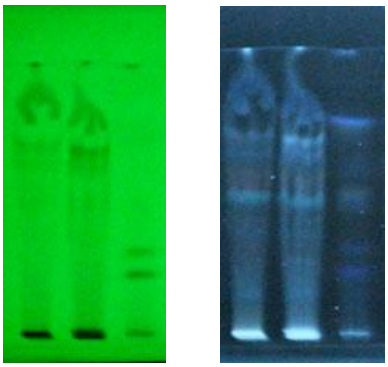
Chromatograph was performed on 10 x 10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel 60F254 stored in a dessicator, application was done by Hamilton microsyringe, mounted on a Linomat V applicator. Spotting [10µl] each was done on the TLC plate, ascending development of the plate, migration distance 80 mm was performed at 25 ± 2^0C with Toluene: ethyl acetate: formic acid::45:5:1 as a mobile phase in a camag chamber previously saturated for 30 mins[15].After development the plates were dried at 60 C in an oven for 5 minutes. Densitometric scanning with WinCATS software at 254 nm and 366 nm was performed and chromatogram was recorded.

RESULTS

The chromatograms are shown in the Fig 1a-f. It is evident from the Table 1 that in 10 µl of petroleum ether extract of dried fruit and fresh leaves of *Coriandrum sativum*, there are 9, 10 and 5 spots respectively. Out of 9 components for soxhlation dried fruit with Rf values 0.57, 0.73 and 0.84 were found to be predominant as the percentage area was found to be 9.25 %, 15.07% and 24.40%. Out of 10 components for maceration dried fruit with Rf values 0.17 and 0.78 were found to be predominant as the percentage area was found to be 5.06 % and 23.95 %. Out of 5 components for maceration fresh leaves with Rf values 0.25,0.34 and 0.82 were found to be predominant as the percentage area was found to be 33.51 %, 21.58 % and 5.63%.
It is evident from the Table 2 that in 10 µl of benzene extract of dried fruit and fresh leaves of *C.sativum*, there are 9, 7 and 10 spots respectively. Out of 9 components for soxhlation dried fruit with Rf values 0.24, 0.51 and 0.81 were found to be predominant as the percentage area was found to be 15.76 %, 7.52% and 6.58%. Out of

7 components for maceration dried fruit with Rf values 0.23,0.33,0.40,0.51,0.7 and 0.79 were found to be predominant as the percentage area was found to be 7.97%,5.89 %,5.26 %,10.43 %,12.86 % and 48.32 %. Out of 10 components for maceration fresh leaves with Rf values 0.23,0.5, and 0.73 were found to be predominant as the percentage area was found to be 10.75 %, 6.18 % and 13.76 %.

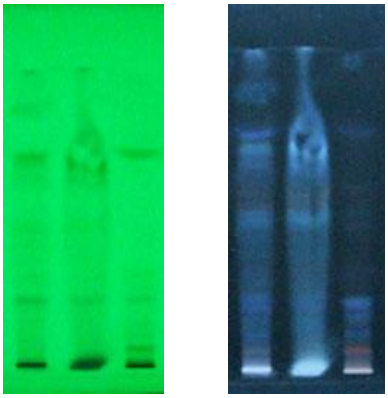
Figure 1 a: HPTLC chromatogram of petroleum ether extracts of *C.sativum* dried fruits [Soxhlation & Maceration] and fresh leaves[Maceration].



254 nm 366 nm

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

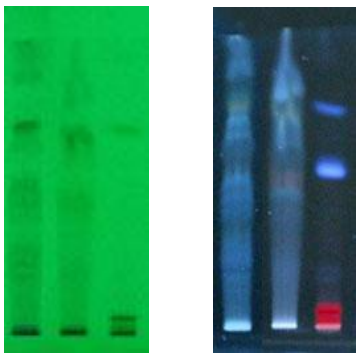
Figure 1 b: HPTLC chromatogram of benzene extracts of *C.sativum* dried fruits[Soxhlation & Maceration] and fresh leaves[Maceration].



254 nm . 366 nm

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

Figure 1 c: HPTLC chromatogram of chloroform extracts of *C.sativum* dried fruits[Soxhlation & Maceration] and fresh leaves[Maceration].



254 nm 366 nm

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

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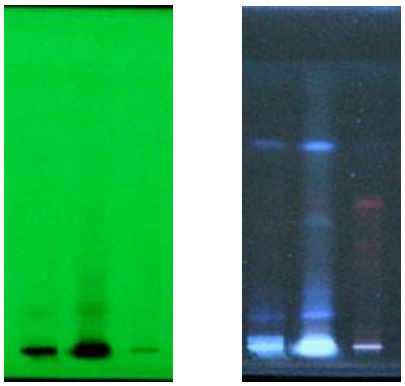
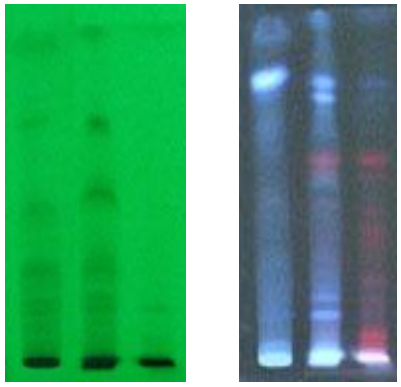
Figure 1 d: HPTLC chromatogram of methanol extracts of *C.sativum* dried fruits[Soxhlation & Maceration] and fresh leaves[Maceration].



254 nm 366 nm

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

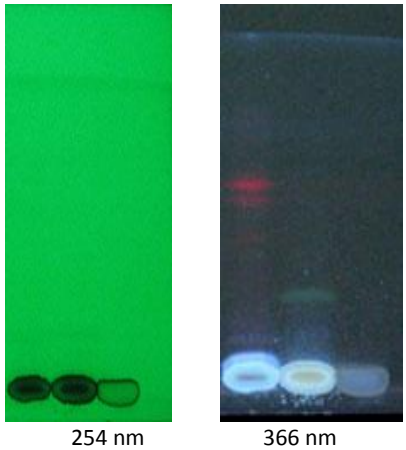
Figure 1 e: HPTLC chromatogram of ethanol extracts of *C.sativum* dried fruits[Soxhlation & Maceration] and fresh leaves[Maceration].



254 nm 366 nm

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

Figure 1 f: HPTLC chromatogram of water extracts of *C.sativum* dried fruits[Soxhlation & Maceration] and fresh leaves[Maceration].



254 nm 366 nm

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

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Table1: Peak list and Rf value of the petroleum ether extracts of *C.sativum* dried fruits [Soxhlation & Maceration] and fresh leaves[Maceration].

Sl.No.	Track 1		Track 2		Track 3	
	Max Rf	Area %	Max Rf	Area %	Max Rf	Area %
1	0.10	1.28	0.09	4.11	0.12	2.36
2	0.17	1.82	0.14	2.86	0.25	33.51
3	0.41	3.67	0.17	5.06	0.34	21.58
4	0.42	2.15	0.29	3.02	0.82	5.63
5	0.57	9.25	0.38	2.14	0.86	1.58
6	0.61	7.83	0.44	1.78		
7	0.73	15.07	0.56	4.92		
8	0.80	24.40	0.78	23.95		
9	0.9	5	0.85	0.4		
10			0.92	0.84		

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

Table 2: Peak list and Rf value of the benzene extracts of *C.sativum* dried fruits[Soxhlation & Maceration] and fresh leaves[Maceration].

Sl.No.	Track 1		Track 2		Track 3	
	Max Rf	Area %	Max Rf	Area %	Max Rf	Area %
1	0.09	2.71	0.23	7.97	0.15	2.78
2	0.24	15.76	0.33	5.89	0.23	10.75
3	0.33	3.87	0.40	5.26	0.32	3.90
4	0.40	1.75	0.51	10.43	0.38	2.65
5	0.44	3.22	0.70	12.86	0.43	1.76
6	0.51	7.52	0.79	48.32	0.50	6.18
7	0.67	1.76	0.95	1.92	0.68	4.64
8	0.69	2.10			0.73	13.76
9	0.81	6.58			0.85	2.99
10					0.88	4.43

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

Table 3: Peak list and Rf value of the chlorofrom extracts of *C.sativum* dried fruits[Soxhlation & Maceration] and fresh leaves[Maceration].

Sl.No.	Track 1		Track 2		Track 3	
	Max Rf	Area %	Max Rf	Area %	Max Rf	Area %
1	0.03	14.55	0.08	6.28	0.08	3.85
2	0.08	4.61	0.16	4.70	0.16	2.97
3	0.12	5.81	0.27	2.96	0.27	2.36
4	0.22	9.17	0.51	1.85	0.39	0.73
5	0.27	4.39	0.57	8.14	0.53	16.29
6	0.32	5.61	0.70	3.63	0.74	27.80
7	0.49	1.18	0.77	18.84	0.81	3.04
8	0.79	15.35	0.92	1.66	0.94	0.86
9			0.94	0.76		
10						

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

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Table 4: Peak list and Rf value of the methanol extracts of *C.sativum* dried fruits [Soxhlation & Maceration] and fresh leaves[Maceration].

Sl.No.	Track 1		Track 2		Track 3	
	Max Rf	Area %	Max Rf	Area %	Max Rf	Area %
1	0.11	2.5	0.08	3.47	0.16	3.83
2	0.23	34.37	0.16	4.06	0.23	11.64
3	0.32	22.98	0.23	12.76	0.31	4.55
4	0.53	3.45	0.32	7.06	0.36	0.73
5	0.78	7.19	0.51	14.82	0.50	8.12
6	0.84	1.48	0.64	0.82	0.72	20.82
7			0.69	1.43	0.83	1.05
8			0.77	26.95		
9			0.91	0.61		
10						

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

Table 5: Peak list and Rf value of the ethanol extracts of *C.sativum* dried fruits[Soxhlation & Maceration] and fresh leaves[Maceration].

Sl.No.	Track 1		Track 2		Track 3	
	Max Rf	Area %	Max Rf	Area %	Max Rf	Area %
1	0.12	4.38	0.14	4.74	0.16	4.28
2	0.20	11.01	0.18	6.53	0.24	10.53
3	0.29	18.73	0.22	3.89	0.29	7.43
4	0.43	1.08	0.27	6.25	0.32	5.38
5	0.55	17.76	0.31	5.64	0.43	10.86
6	0.77	21.61	0.34	5.96	0.53	37.17
7			0.43	3.42	0.67	4.01
8			0.50	3.45	0.72	3.17
9			0.55	11.97		
10			0.72	2.32		
11			0.79	9.58		
12			0.87	0.37		
13			0.90	0.18		
14			0.98	0.23		

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

Table 6: Peak list and Rf value of the water extracts of *C.sativum* dried fruits[Soxhlation & Maceration] and fresh leaves[Maceration].

Sl.No.	Track 1		Track 2		Track 3	
	Max Rf	Area %	Max Rf	Area %	Max Rf	Area %
1	0.10	6.62	0.04	89.94	0.03	93.19
2	0.16	6.16	0.07	0.53	0.08	0.94
3	0.25	8.14	0.11	0.77	0.10	0.52
4	0.29	10.79	0.17	3.36	0.18	2.66
5	0.43	4.40	0.25	1.18	0.21	0.7
6	0.49	6.13	0.81	1.67	0.8	1.99
7	0.53	10.84	0.96	2.54		
8	0.75	11.71				
9	0.96	0.96				
10						

Track 1: Soxhlation extract of dried fruit; Track 2: Maceration extract of dried fruit; Track 3: Maceration extract of fresh leaves.

It is evident from the Table 3 that in 10 µl of chloroform extract of dried fruit and fresh leaves of *C.sativum*, there are 8, 9 and 8 spots respectively. Out of 8 components for soxhlation dried fruit with Rf values 0.03,0.12,0.22,0.32 and 0.79 were found to be predominant as the percentage area was found to be 14.55 %,

5.81 %, 9.17%,5.61% and 15.35%. Out of 9 components for maceration dried fruit with Rf values 0.08,0.57 and 0.77 were found to be predominant as the percentage area was found to be 6.28 %, 8.14% and 18.84 %. Out of 8 components for maceration fresh leaves with Rf values 0.53 and 0.74 were found to be predominant as the percentage area was found to be 16.29 % and 27.80 %.

It is evident from the Table 4 that in 10 µl of methanol extract of dried fruit and fresh leaves of *C. sativum*, there are 6, 9 and 7 spots respectively. Out of 6 components for soxhlation dried fruit with Rf values 0.23,0.32 and 0.79 were found to be predominant as the percentage area was found to be 34.37 %, 22.98 % and 7.19 %. Out of 9 components for maceration dried fruit with Rf values 0.23,0.32,0.51 and 0.77 were found to be predominant as the percentage area was found to be 12.76 %, 7.06 %, 14.82 % and 26.95 %. Out of 7 components for maceration fresh leaves with Rf values 0.23,0.5 and 0.72 were found to be predominant as the percentage area was found to be 11.64 %, 8.12 % and 20.82 %.

It is evident from the Table 5 that in 10 µl of ethanol extract of dried fruit and fresh leaves of *C.sativum*, there are 6, 14 and 8 spots respectively. Out of 6 components for soxhlation dried fruit with Rf values 0.22,0.29,0.55 and 0.77 were found to be predominant as the percentage area was found to be 11.01 %, 18.73 %, 17.76 % and 21.61 %. Out of 14 components for maceration dried fruit with Rf values 0.18,0.27,0.31,0.34,0.55and 0.79 were found to be predominant as the percentage area was found to be 6.53 %, 6.25%, 5.64%, 5.96 %, 11.97% and 9.58 %. Out of 8 components for maceration fresh leaves with Rf values 0.24,0.29,0.32,0.43 and 0.53 were found to be predominant as the percentage area was found to be 10.53%, 7.43%,5.38%,10.86% %, and 37.17%.

It is evident from the Table 6 that in 10 µl of aqueous extract of dried fruit and fresh leaves of *C.sativum*, there are 9, 7 and 6 spots respectively. Out of 9 components for soxhlation dried fruit with Rf values 0.10,0.16,0.25,0.29,0.49,0.53 and 0.75 were found to be predominant as the percentage area was found to be 6.62%, 6.16%,8.14%,10.79% %, 6.13%, 10.84% and 11.71%. Out of 7 components for maceration dried fruit with Rf value 0.04 was found to be predominant as the percentage area was found to be 89.94 %. Out of 6 components for maceration fresh leaves with Rf value 0.03 was found to be predominant as the percentage area was found to be 93.19 %.

The remaining components were found to be very less in quantity as the percent area for all the spots were less than 5%.

DISCUSSION

Thus the developed chromatogram will be specific with selected solvent system Toluene: ethyl acetate: formic acid::45:5:1, Rf value and serve the better tool for standardization of the drug.

Characteristic TLC/HPTLC fingerprinting of particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species. Thus the present study will provide sufficient information about therapeutic efficacy of the drug and also in the identification, standardization and quality control of medicinal plant.

CONCLUSION

Herbal medicines are composed of many components and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicines.the results obtained from qualitative evaluation of HPTLC fingerprint images will be helpful in identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of *C.sativum* Linn leaves and dried fruit can be used as a reference for the identification and quality control of the drug.

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