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HPTLC analysis of the essential oil from *Pimenta dioica* leaf

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ABSTRACT

The present study was done with an aim to standardize the volatile oil obtained from the leaf of *Pimenta dioica* (Linn.) Merill, belonging to the family Myrtaceae, commonly known as "Allspice", by HPTLC analysis. The leaves of *P. dioica* is traditionally being used as a dental analgesic. The present investigation reports the seasonal variation in the content of eugenol, in the leaf volatiles of *Pimenta dioica* thereby giving insight into the most favorable month for the collection of the drug. Volatile oils are primarily composed of terpenes, the composition of which may alter depending upon the availability of sunlight. The results reveal that the oil collected in April and July showed good content of eugenol.

INTRODUCTION

Pimenta dioica (Linn.) Merill. Family: Myrtaceae, has been used as an important spice since time immemorial. The drug has derived the name "Allspice" since its aroma resembles the aroma of spices such as Clove, Nutmeg and Cinnamon (Neal, 1965; Weiss, 2002). In India, the leaves of Pimenta are used to flavor rice which gives it a typical aroma. Allspice is considered as a very important spice in the meat industry which utilizes the powder of the berries for tenderizing of meat (Seidemann, 2005; Sharma, 2003). The essential oil of berries of *Pimenta dioica* has been reported to contain the following, limonene, 1,8 cineole, terpinolene, β-caryophyllene, β-selinene and methyl eugenol. The Jamaican Pimento leaf oil contains eugenol, methyl eugenol, myrcene and β-caryophyllene (Tucker et al., 1991). Another study carried out on Pimento leaf oil in Jamaica showed the presence of Eugenol, Methyl Eugenol and β-caryophyllene as the main compounds whereas Myrcene was found in trace amounts. The leaf oil is considered to be inferior to the berry oil (Jirovetz et al., 2007). The therapeutic properties of the allspice berry oils are anesthetic, analgesic, antimicrobial, antioxidant, antiseptic, acaricidal, carminative, muscle relaxant, rubefacient, stimulant and tonic. Pimento oil can be helpful for the digestive system, for cramp, flatulence, indigestion and nausea. Further, the essential oils can help in cases of depression, nervous exhaustion, tension, neuralgia and stress and is used as natural repellent. The essential *P. dioica* leaf and fruit oil is also used in perfumes, aftershaves and commercial food flavoring (Seidemann, 2005; Sharma, 2003). The present work was carried out to standardize the phytoconstituent in allspice leaf volatile oil via HPTLC analysis. Standardization of eugenol was done in the leaf volatiles obtained in summer and monsoon seasons from South Canara district, Karnataka, India.

MATERIALS AND METHODS

Plant material

Procurement of leaves

The leaves used for the investigation were collected from a private garden at a place called Puttur in Dakshina Kannada district, Karnataka in the months of Jan-May (Summer) and June-October (Monsoon). The leaves were shade dried, powdered and stored in air tight containers to prevent the loss of volatile oil till further use. A voucher specimen PD06-01 is stored with us.

Identification

The plant under investigation was identified with the help of flora and also with the help of two local taxonomists, viz., Prof. Rajagopal, from Mahatma Gandhi Memorial College, Udupi and

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Prof. Gopalakrishna Bhat, from Poorna Prajna College, Udupi, Karnataka.

Extraction of the oil

The leaf powder was hydrodistilled in a Clevenger's apparatus to yield yellowish brown oil. The essential oils were designated the code AOS1-AOS5 for oils collected in Summer (Jan- May) and AOM1-AOM5 for oil collected in Monsoon (June-October) respectively. Yield of the oil varied from 1.5-2.2% w/v.

HPTLC analysis

Eight microlitres of the oil samples diluted in ethanol and two microlitre of eugenol were spotted on pre-coated silica gel TLC plate (E. Merck) of dimension (20 X 10cm) after activation at 105°C. The plates were developed in a pre-saturated Twin trough chamber containing the solvent system Toluene: Ethyl acetate (93:7). Developed plate was air dried and scanned under UV 254nm using Camag densitometer and the chromatogram was noted.

RESULTS AND DISCUSSION

Analysis of the summer samples revealed that the sample AOS4 contained more amount of eugenol. In the monsoon sample, AOM2 showed good results in terms of eugenol concentration. Seasonal variation amongst chemical constituents is deemed to be very important factor in commerce, since the utilization of the drug depend upon the amount of chemical constituents.

The findings clearly show that environmental and geographical conditions influence the content of essential oil and their composition in volatile oil containing plants.

Pimenta dioica plant seems to show a high content of eugenol in the leaves during monsoon season. From the present study we can conclude that in the region of study, the most efficient month for collection of leaves is April and July respectively. More detailed studies are however required to confirm the variation in the presence of other compounds also, in volatile oil samples collected during different above mentioned months in Summer and Monsoon respectively.

CONCLUSION

The present study gives us an insight into the variation in chemical composition of leaf volatiles of the plant, *Pimenta dioica* collected during two main seasons in South Canara District of Karnataka Region of India, Summer and Monsoon. The composition of leaf volatiles would have showed variation due to the change in the intensity of sunlight available during the above mentioned seasons. Commercialization of eugenol, an important medicinal chemical constituent obtained from leaf volatiles of *P. dioica* would be easier if the month of collection of leaves, where eugenol content is more is known. The present study has standardized the content of eugenol in the leaf volatiles collected in the different seasons, however; more extensive studies on variation of other constituents are planned.

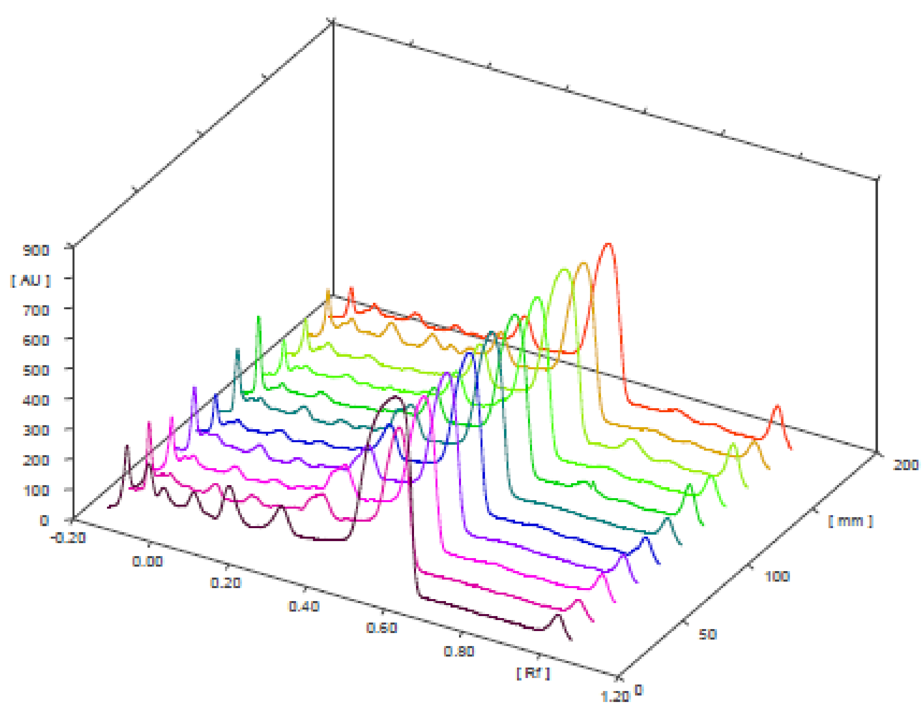


Fig. 1: HPTLC densitometric scan of leaf volatile of *Pimenta dioica*.

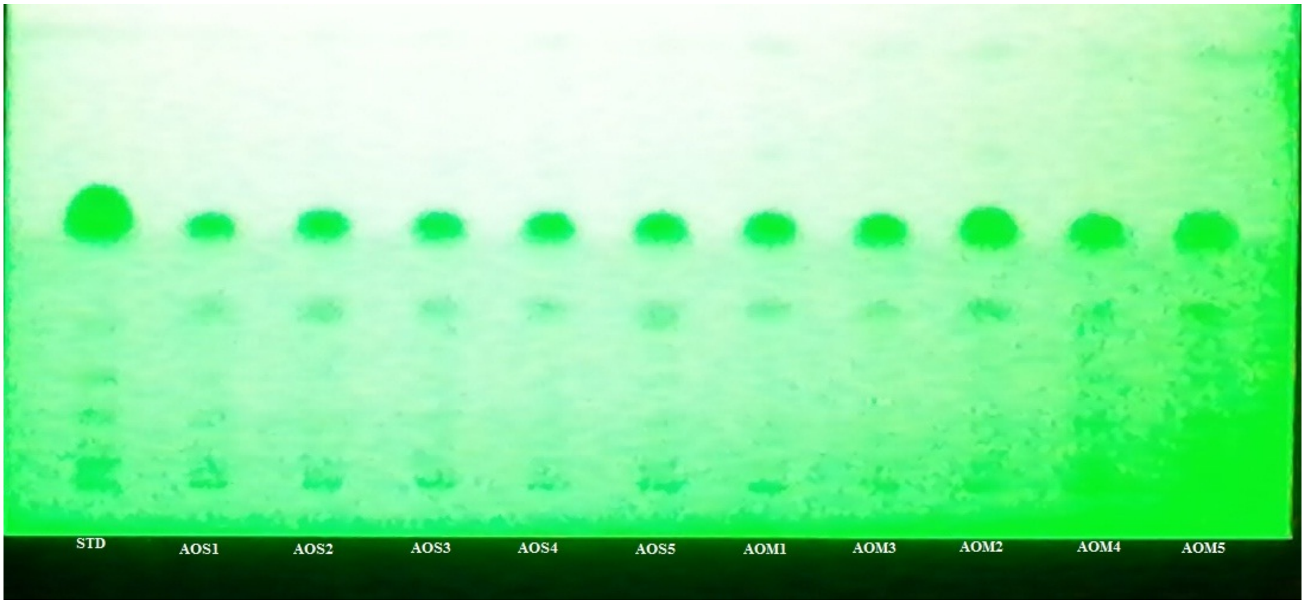
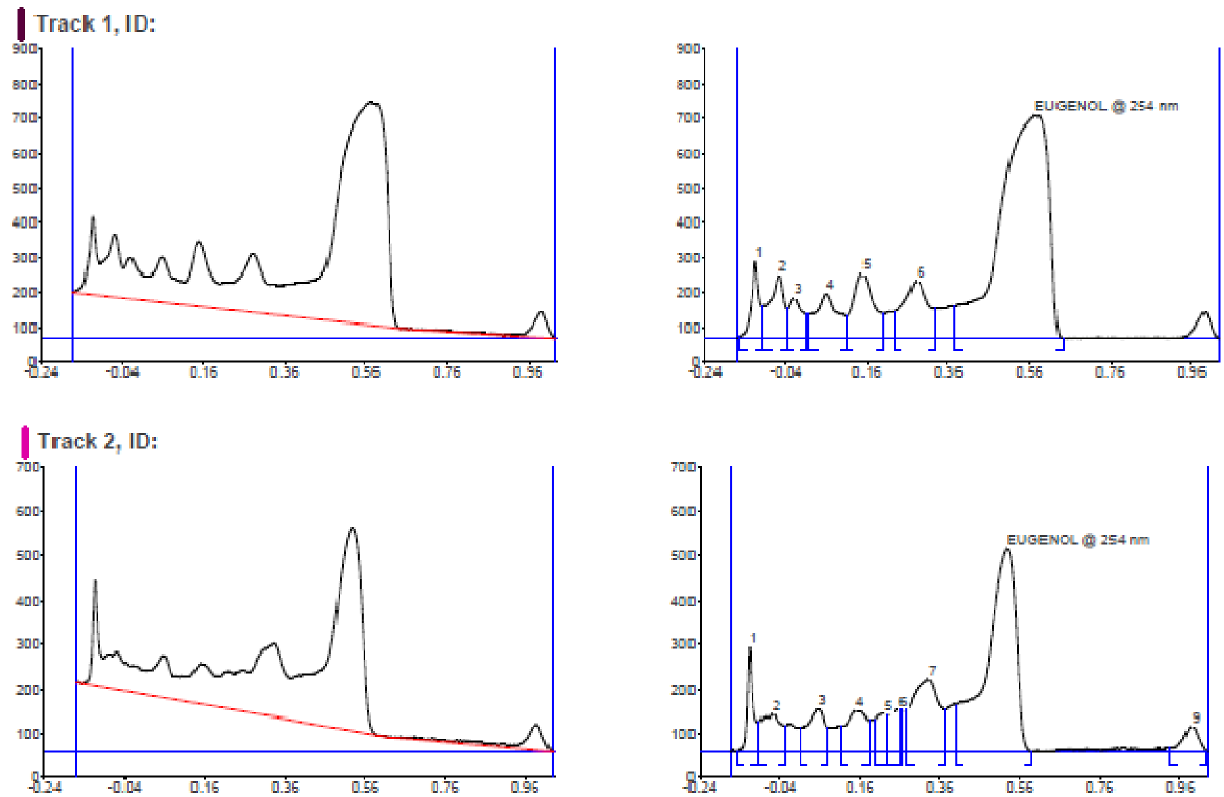
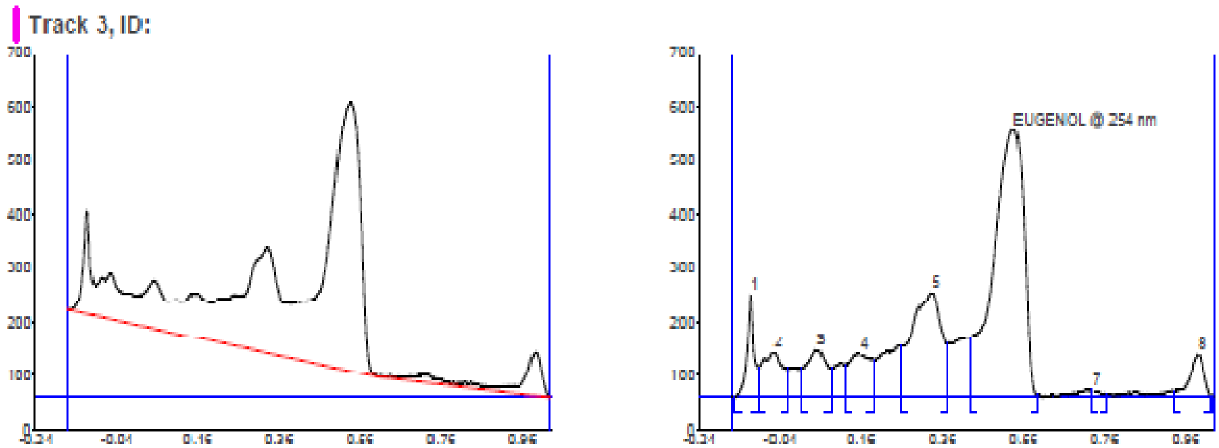


Fig. 2: HPTLC chromatogram of the volatile oil of *Pimenta dioica* collected in summer and monsoon respectively.



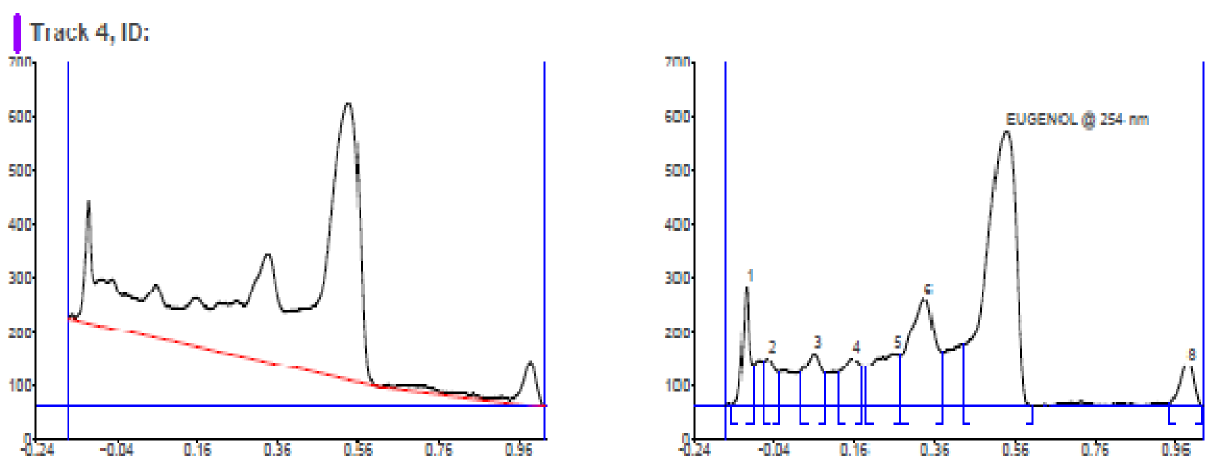
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.14	0.2	-0.11	234.0	17.18	-0.09	65.7	2583.2	5.11	unknown *
2	-0.09	66.4	-0.06	83.9	6.16	-0.03	57.2	2951.0	5.84	unknown *
3	0.01	53.5	0.06	95.1	6.99	0.09	53.5	3252.6	6.44	unknown *
4	0.11	56.2	0.15	91.5	6.72	0.19	69.7	3542.6	7.01	unknown *
5	0.20	70.6	0.23	84.9	6.24	0.23	82.4	1698.2	3.36	unknown *
6	0.23	82.5	0.26	93.7	6.88	0.27	92.4	1961.0	3.88	unknown *
7	0.28	93.3	0.33	164.0	12.04	0.38	94.7	8051.2	15.93	unknown *
8	0.40	104.5	0.53	456.7	33.53	0.59	0.1	24949.9	49.36	EUGENOL
9	0.94	7.4	0.99	58.0	4.26	1.03	1.0	1554.0	3.07	unknown *

Fig. 3: Chromatogram peak area table for AOS1.



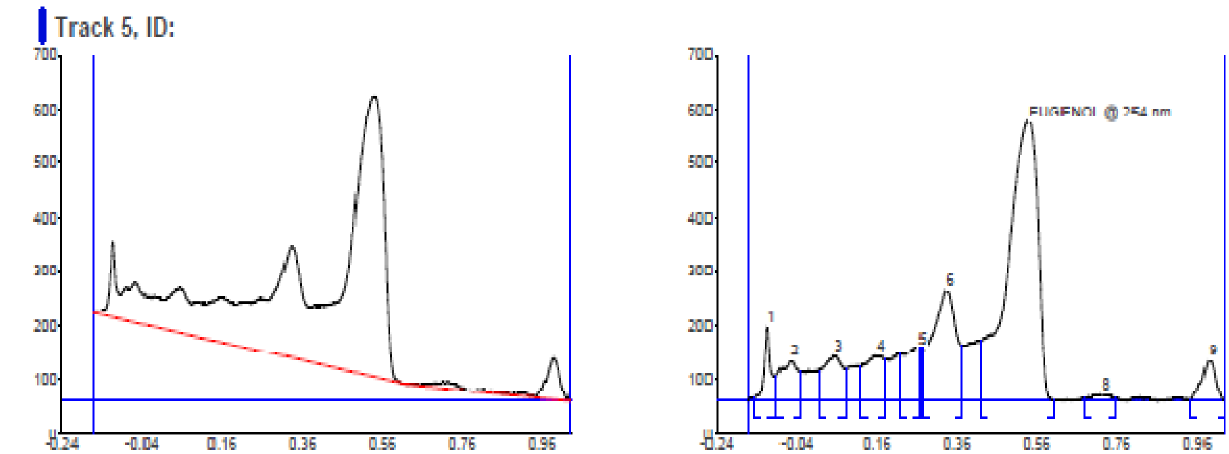
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.15	0.3	-0.11	190.8	15.51	-0.09	56.4	2476.7	4.70	unknown *
2	-0.09	56.5	-0.05	83.2	6.76	-0.02	52.9	3002.5	5.70	unknown *
3	0.01	54.1	0.05	87.6	7.12	0.09	56.0	3316.5	6.29	unknown *
4	0.12	60.4	0.16	81.5	6.62	0.19	69.7	3344.1	6.35	unknown *
5	0.26	95.6	0.34	195.0	15.85	0.38	100.6	10584.8	20.09	unknown *
6	0.43	111.7	0.54	498.5	40.51	0.60	4.1	27573.3	52.33	EUGENOL
7	0.73	13.8	0.74	14.0	1.14	0.77	5.2	259.4	0.49	unknown *
8	0.93	11.9	1.00	79.8	6.49	1.03	0.7	2131.7	4.05	unknown *

Fig. 4: Chromatogram peak area table for AOS2.



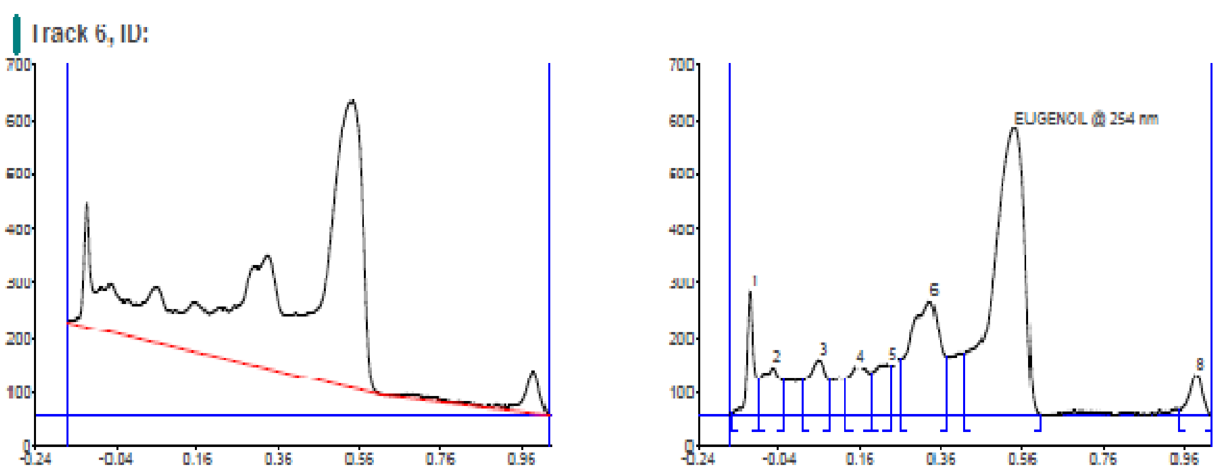
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.14	5.1	-0.11	226.5	16.18	-0.09	77.4	2869.5	5.19	unknown *
2	-0.07	83.8	-0.05	90.3	6.45	-0.02	63.9	2122.6	3.84	unknown *
3	0.03	63.7	0.06	99.3	7.09	0.09	62.2	3127.0	5.65	unknown *
4	0.12	64.5	0.16	89.4	6.39	0.18	76.7	3020.3	5.46	unknown *
5	0.19	74.7	0.26	99.3	7.10	0.28	96.6	4864.8	8.79	unknown *
6	0.28	96.4	0.34	200.4	14.32	0.38	101.0	9534.7	17.23	unknown *
7	0.43	114.8	0.54	514.6	36.76	0.61	0.1	27821.1	50.28	EUGENOL
8	0.95	8.8	1.00	80.1	5.72	1.03	3.3	1972.2	3.56	unknown *

Fig 5: Chromatogram peak area table for AOS3.



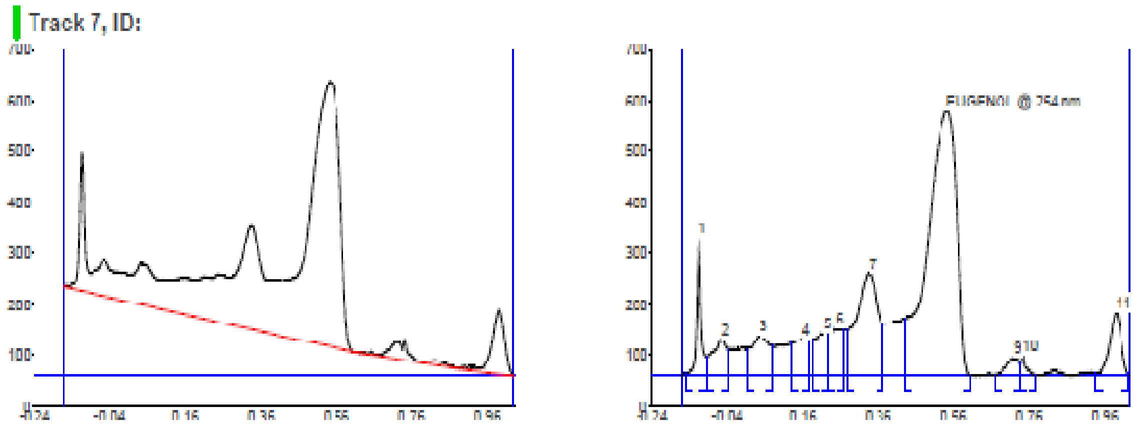
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.14	7.1	-0.11	138.9	10.76	-0.09	45.7	1712.0	3.15	unknown *
2	-0.09	46.4	-0.05	74.1	5.74	-0.03	53.6	2454.1	4.51	unknown *
3	0.02	57.3	0.06	83.0	6.43	0.09	59.8	3087.5	5.68	unknown *
4	0.12	65.7	0.17	83.5	6.47	0.18	77.4	3121.9	5.74	unknown *
5	0.22	85.0	0.27	98.8	7.65	0.27	98.1	3046.4	5.60	unknown *
6	0.28	97.7	0.34	205.9	15.95	0.38	103.0	9311.7	17.12	unknown *
7	0.43	113.1	0.54	517.9	40.13	0.61	2.4	29345.9	53.94	EUGENOL
8	0.68	4.3	0.73	13.0	1.01	0.76	4.2	461.6	0.85	unknown *
9	0.95	1.4	1.00	75.6	5.86	1.03	0.0	1860.2	3.42	unknown *

Fig 6: Chromatogram peak area table for AOS4.



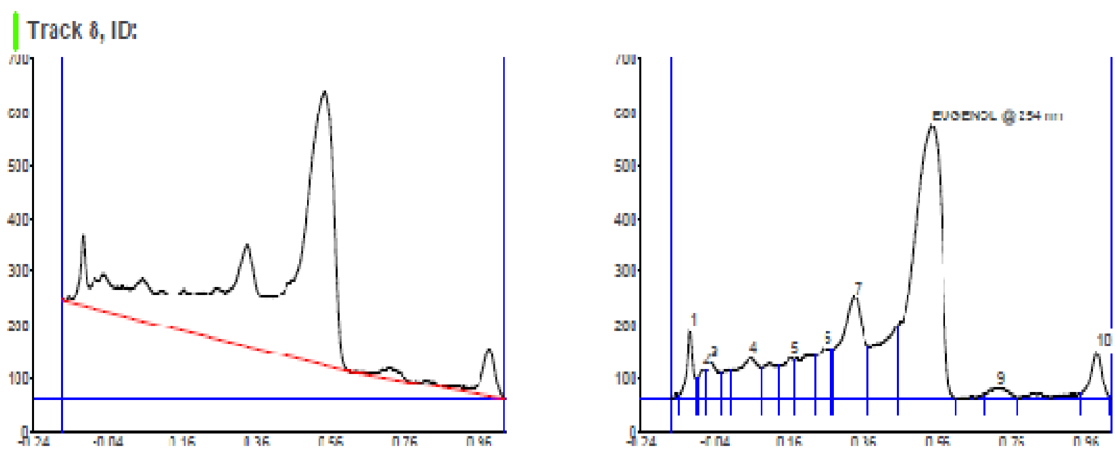
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.15	5.4	-0.11	225.8	16.12	-0.09	65.8	2905.2	4.89	unknown *
2	-0.09	65.6	-0.05	86.2	6.15	-0.03	61.3	2953.4	4.98	unknown *
3	0.02	62.6	0.06	100.3	7.16	0.09	63.3	3499.4	5.90	unknown *
4	0.13	66.1	0.15	89.5	6.39	0.19	75.8	3387.8	5.71	unknown *
5	0.19	76.4	0.23	92.5	6.60	0.24	88.6	2700.2	4.55	unknown *
6	0.26	100.6	0.34	206.5	14.74	0.38	105.6	11619.7	19.58	unknown *
7	0.42	112.8	0.55	523.6	37.38	0.61	0.9	30453.7	51.30	EUGENOL
8	0.95	10.7	1.00	76.2	5.44	1.03	0.0	1839.6	3.10	unknown *

Fig. 7: Chromatogram peak area table for AOS5.



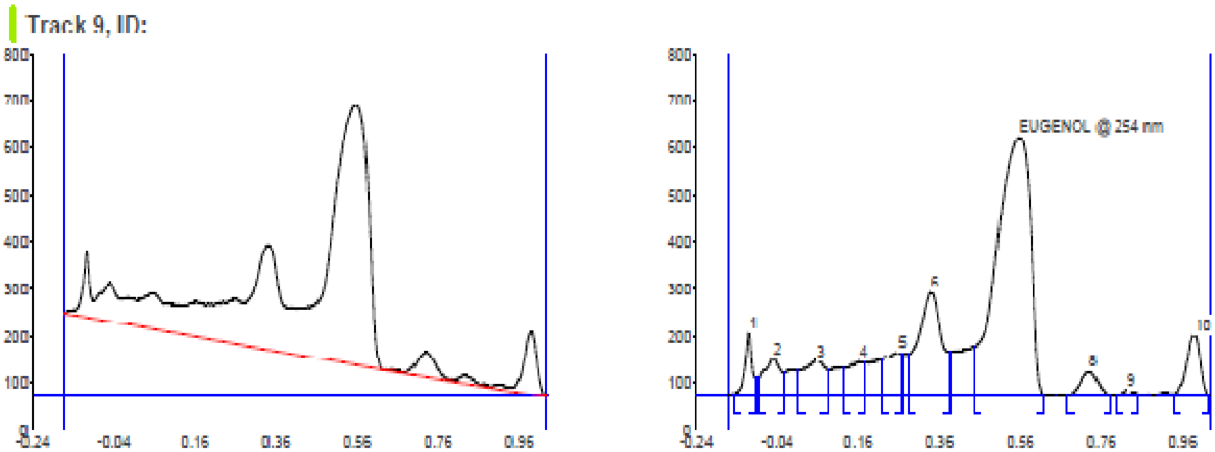
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.15	4.6	-0.11	270.2	16.99	-0.09	37.9	2667.5	4.74	unknown *
2	-0.09	38.1	-0.05	71.1	4.47	-0.03	52.5	1885.5	3.35	unknown *
3	0.01	52.8	0.05	78.0	4.90	0.08	58.7	2976.7	5.29	unknown *
4	0.13	63.4	0.16	72.6	4.56	0.18	70.6	1937.0	3.44	unknown *
5	0.19	71.5	0.22	82.7	5.20	0.23	81.8	2203.7	3.92	unknown *
6	0.23	82.1	0.25	93.0	5.85	0.27	91.1	2425.9	4.31	unknown *
7	0.28	91.5	0.34	200.8	12.62	0.37	102.7	8354.3	14.84	unknown *
8	0.43	112.5	0.55	520.9	32.74	0.61	0.1	29779.3	52.91	EUGENOL
9	0.67	3.0	0.73	35.0	2.20	0.74	20.4	852.9	1.52	unknown *
10	0.74	22.8	0.75	41.1	2.58	0.78	1.6	376.7	0.67	unknown *
11	0.94	5.1	1.00	125.3	7.87	1.03	6.2	2827.2	5.02	unknown *

Fig 8: Chromatogram peak area table for AOM1.



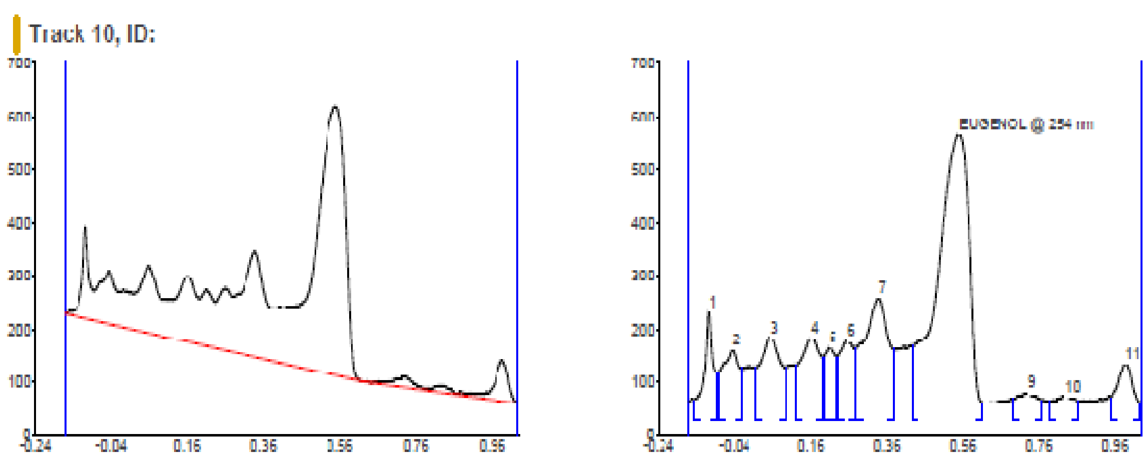
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.13	6.7	-0.10	129.8	9.77	-0.09	39.1	1549.0	3.01	unknown *
2	-0.09	39.6	-0.07	56.9	4.29	-0.06	53.9	756.5	1.47	unknown *
3	-0.06	54.4	-0.05	68.6	5.16	-0.02	49.7	1692.3	3.29	unknown *
4	0.01	53.6	0.06	78.3	5.90	0.09	58.0	3388.0	6.59	unknown *
5	0.13	61.6	0.17	79.2	5.96	0.18	72.5	2000.0	3.89	unknown *
6	0.23	79.7	0.26	96.9	7.29	0.28	93.1	2814.2	5.48	unknown *
7	0.28	93.3	0.34	191.9	14.45	0.38	99.8	8452.5	16.45	unknown *
8	0.45	139.1	0.55	515.9	38.85	0.61	0.1	27878.1	54.25	EUGENOL
9	0.69	7.3	0.72	20.7	1.56	0.78	1.9	718.4	1.40	unknown *
10	0.95	8.2	0.99	90.0	6.77	1.03	6.4	2141.3	4.17	unknown *

Fig. 9: Chromatogram peak area table for AOM3.



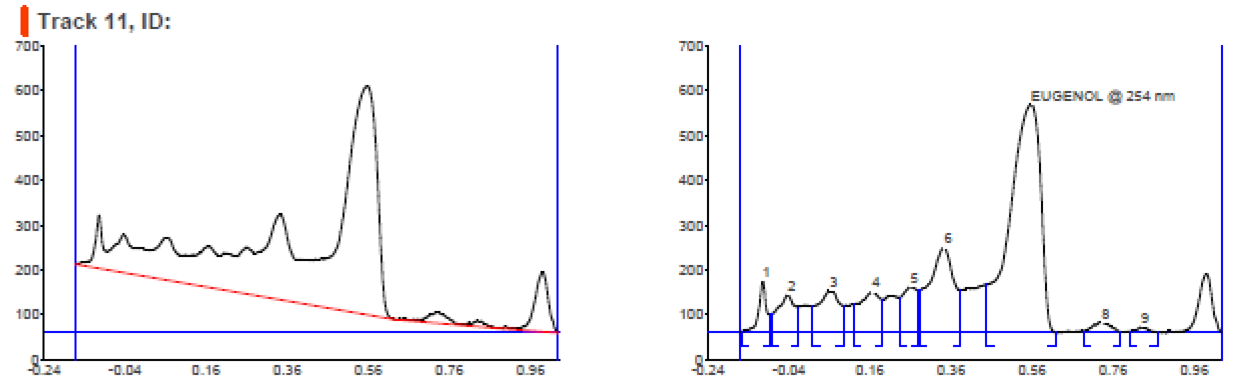
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.14	9.0	-0.10	137.0	9.50	-0.09	39.4	1844.8	2.98	unknown *
2	-0.09	39.6	-0.05	82.9	5.75	-0.02	51.3	2645.9	4.28	unknown *
3	0.02	55.9	0.06	76.2	5.28	0.09	57.9	3043.5	4.92	unknown *
4	0.13	60.7	0.17	74.3	5.16	0.18	72.6	2270.6	3.67	unknown *
5	0.22	79.0	0.26	95.7	6.64	0.27	91.3	2825.7	4.57	unknown *
6	0.29	91.3	0.34	222.1	15.40	0.39	97.0	9692.9	15.67	unknown *
7	0.45	106.3	0.56	552.0	38.29	0.62	0.3	34239.4	55.35	EUGENOL
8	0.68	3.5	0.73	53.2	3.69	0.79	3.6	1761.9	2.85	unknown *
9	0.80	4.5	0.83	16.3	1.13	0.86	6.3	398.9	0.64	unknown *
10	0.94	8.8	0.99	132.1	9.17	1.03	3.3	3133.4	5.07	unknown *

Fig 10: Chromatogram peak area table for AOM2.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.14	7.1	-0.10	172.2	11.07	-0.08	56.6	2426.0	3.95	unknown *
2	-0.08	57.3	-0.04	100.5	6.46	-0.02	65.3	3029.8	4.93	unknown *
3	0.02	67.3	0.06	125.1	8.04	0.10	68.6	4648.5	7.56	unknown *
4	0.12	72.4	0.17	123.2	7.91	0.19	87.2	4433.8	7.21	unknown *
5	0.20	87.2	0.21	105.8	6.80	0.23	86.8	2502.0	4.07	unknown *
6	0.24	87.5	0.26	116.6	7.49	0.28	104.3	3186.7	5.18	unknown *
7	0.28	104.7	0.34	197.4	12.69	0.38	100.6	8899.7	14.48	unknown *
8	0.43	108.5	0.55	506.4	32.55	0.61	2.3	29387.7	47.80	EUGENOL
9	0.70	8.3	0.73	18.9	1.22	0.77	6.4	642.7	1.05	unknown *
10	0.79	3.7	0.83	14.0	0.90	0.87	4.2	449.5	0.73	unknown *
11	0.95	11.6	0.99	75.9	4.88	1.03	0.9	1869.0	3.04	unknown *

Fig. 11: Chromatogram peak area table for AOM4.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.15	3.2	-0.10	117.5	9.57	-0.08	40.6	1672.1	3.22	unknown *
2	-0.08	40.8	-0.04	84.6	6.89	-0.02	57.8	2635.1	5.08	unknown *
3	0.02	59.5	0.06	94.1	7.66	0.10	60.2	3599.5	6.94	unknown *
4	0.12	63.9	0.17	91.9	7.48	0.19	74.6	3485.2	6.72	unknown *
5	0.24	79.8	0.26	103.5	8.43	0.28	94.0	2836.7	5.47	unknown *
6	0.29	94.2	0.35	189.9	15.47	0.38	96.1	8443.9	16.28	unknown *
7	0.45	107.2	0.56	508.9	41.46	0.62	1.1	28090.6	54.14	EUGENOL
8	0.69	3.8	0.74	24.3	1.98	0.78	2.8	811.0	1.56	unknown *
9	0.80	2.4	0.83	13.0	1.06	0.87	1.2	306.5	0.59	unknown *

Fig. 12: Chromatogram peak area table for AOM5.

Table. 1: Peak Area table for Summer and Monsoon sample.

SAMPLE	PEAK AREA	% AREA
AOS1	24949.9	49.36
AOS2	27573.3	52.33
AOS3	27821.1	50.28
AOS4	29345.9	53.94
AOS5	30453.7	51.30
AOM1	29779.3	52.91
AOM2	34239.4	55.35
AOM3	27878.1	54.25
AOM4	29387.7	47.80
AOM5	28090.6	54.14

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