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Analysis of Chemical Constituents of the Steam Distillate of *Trachysperum roxburghianum*

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Abstract: The steam distillate of the spice, *T. roxburghianum* seeds was analyzed by GLC and GCMS techniques. Analysis revealed that the constituent of ingredients of the essential oil was different than that of oil obtained from other species like *T. ammi*, *T. stictocarpum,* and *T. bulbocastanum* seeds. GC analysis and preliminary investigation on TLC plate visualized five major compounds along with several minor constituents. This manuscript deals with the identification of these compounds done by GC-MS analysis.

Keywords: *T. roxburghianum*, Apiaceae, Steam Distillate, Characterization, GC-MS Analysis

1. Introduction

Plants in the genus *Trachyspermum* are valued for their medicinal properties [1-7] besides their extensive use as spices in domestic cooking specially in the eastern part of India [8, 12]. Various species of the plants are used in ayurvedic preparations [5, 10]. Amongst the different *Trachyspermum* species [8-12] most of the scientific investigations have been carried out with *T. ammi*, and *T. stictocarpum* [11, 15]. The seed extract of the *T. ammi* exhibits antispasmodic [13] and stimulant activities [13] and is prescribed as house hold remedy for indigestion [10, 11]. Besides, the seeds extract is reported to possess anti-inflammatory [13, 14], anti-bacterial [15], anti-fungal [16-21], insecticidal [22, 23], anti-hyperlipidemic [24, 25] anti-oxidant [21, 27], anti-filarial [26, 28], nematocidal [29, 30], molluscidal activity [31], gastro protective activity [32, 34], anti-aggregatory activity [35], anti-tussive [36], allosteric activity [37] and anti-yeast [38]. The seed extract is also used in pickle, bakery-confectionary industry and cookery ingredients due to its pleasant aroma especially in the India. *T. bulbocastanum* is especially used for preparation of fried rice in Kashmiri disc. The steam volatile part of *T. ammi*, *T. stictocarpum* seeds has been investigated extensively and variations in the chemical constituents are reported in those different varieties [39-42, 45, 46].

The plant is an erect, branched up to 90 cm tall cultivated as minor in north-eastern parts of India. The fruits are ovoid, aromatic cremocrops, 2-3 mm long grayish brown in color, mericarps impressed with distinct ridges and tabular surface and one seeded [47] are in cold weather especially in winter season in India. Several commercial types of the crops are available in the market in different names in accordance to their origin as well as varieties. Some of the best delicious quality comparatively small seeded varieties are produced chiefly in West Bengal. Its use in Indian traditional medicines is also well documented [45]. So far no phytochemical investigation on steam distillate of seeds of *T. roxburghianum* is reported in literature. Given the importance of the seeds as cookery ingredient, food additive and in traditional medicine especially in ayurved preparation, analyze of the steam distillate of *T. roxburghianum* seeds by GC-MS.

2. Experimental Section

2.1. Materials and Methods

T. roxburghianum (syn. *Carum roxburghianum*) seeds were procured from local market Kolkata, India in September 2004. Other chemicals including solvents used were of analytical grades. The GLC analysis were performed with a Chemito Chromatogram using 3% OV-17 column, 40 ml/min N_2 as carrier gas, temperature program 60-240°C @ 4°C/min and a flame ionization detector. The GC-MS analysis were

carried out with GCMS-QP5050A Gas Chromatograph mass spectrometer, Shimadzu using DB-5 (30 m x 0.25 mm) splitting capillary column with a split ratio 50, 0.9 ml/min He as carrier gas and a temperature program 60-240°C and a mass detector. The GCMS data were analyzed by comparing the mass spectral data with those obtained from respective authentic sample as well as library search available in GCMS-QP5050A. The ^{1}H NMR of the essential oil was recorded with a Bruker AV200 (200 MHz in $CDCl_3$) spectrophotometer reveal that absence of any aromatic compounds.

2.2. Preparation of Steam Distillate

The cleaned powdered seed (1kg) along with distilled water (1.5 lit.) was taken in a three necked round bottom flask fitted with a steam generator. The apparatus was assembled for the downward distillation and the distillate obtained by passing steam was condensed and collected in a receiver. Distillation was continued for a period of 2h. The distillate was extracted with ether by usual worked up, and analyzed by GCMS.

3. Results and Discussion

In order to analyze the essential oil composition of the *T. roxburghianum* seeds, it was subjected to steam distillation to obtain the steam distillate in 2.5% w/w yield of the dry seeds. The essential oil was directly analyzed by GLC using temperature programming. Five terpenoids namely 1-limonene, terpineol, *trans* and *cis*-ligustilide and caryophyllene were major constituents as revealed by comparison of GLC data with authentic samples, computer library search and literature knowledge. In addition, the minor constituents as mentioned in the Table 1 were also present in the essential oil and the mass spectral fragmentation patterns of the constituents compared with those available in mass library. The chemical composition of the essential oil of *T. roxburghianum* seeds are summarized in Table 1.

Earlier the essential oil from *T. ammi* seeds have been reported to contain thymol (39.45%), γ-terpene (30.97%) as major constituents along with α and β-pinenes [28]. The presence of cumene and carvone was also reported in some of the varieties [45]. In *T. stictocarpum* medicinally important coumarins such as seselin, bergaptin, dihydropsoralin, α-cadenene, ocimene, carvone, citral, jasmonene, α-terpineol was presented as major constituents [45]. In addition, minor components such as α-pinene and β-pinene, jasmine, jasmonol, dehydrobornol, p-carveol, linalool, lavendulol and isocyclocitranal were present in the essential oil. In contrast essential oil obtained from *T. roxburghianum* does not contain any coumarins though these belong to same species. Moreover the ether extract of seeds of this plant contained oleic acid its oleyl ester as the major products. Along with these products trace amount oleyl ester of linolic, linolenic, plamitic, stearic acid and corresponding free acids in trace amount were also available [41].

All together twenty six peaks appeared in GCMS (Figure 1). Out of these, five compounds appeared as major constituents. The GCMS data of both major and minor peaks are summarized in Table 1.

Table 1. *Results of GCMS analysis of steam distillate of T. roxburghianum seeds.*

R_t.(min)	Rel. abund (%)	Mol. Wt.	Name of comp.	Molecular ions and their fragmentations m/z (%)
9.32	Very trace	136/$C_{10}H_{16}$	Sabinene	136 [M^+] (9.42), 121 (3.41), 107 (2.2), 93 (100), 77 (43.80), 65 (10.27), 55 (5.52), 41 (49.67).
11.10	Trace	136/$C_{10}H_{16}$	α-Terpinene	136 [M^+] (35.1), 121 (82.21), 105 (19.74), 93 (100), 77 (44.81), 65 (13.62), 58 (6.19), 41 (44.22).
11.40	Trace	152/ $C_{10}H_{16}O$	Trans-3-caren-2-ol	152 [M^+] (not appeared), 134 (21.42), 119 (100), 105 (8.36), 91 (40.27), 77 (23.22), 65 (13.52), 41 (22.36).
11.66	26.12	136/$C_{10}H_{16}$	1-Lemonene	136 [M^+] (9.7), 121 (12.9), 107 (13.4), 93 (52.2), 79 (33.00), 69 (100), 53 (26.6), 41 (36.4).
12.77	3.7	136/ $C_{10}H_{16}$	γ-Terpenine	136 [M^+] (23.85), 121 (23.1), 105 (10.3), 93 (100), 77 (40.6), 65 (11.2), 51 (11.8), 43 (42.6).
13. 30	4.6	154/$C_{10}H_{18}O$	Sabinene hydrate	154 [M^+] (1.2), 136 (5.1), 121 (10.2), 111 (14.1), 93 (30.3), 71 (54.8), 55 (25.0), 43 (100).
13.86	Trace	136/$C_{10}H_{16}$	Terpinolene	136 [M^+] (54.50), 121 (64.9), 105 (23.0), 93 (100), 79 (51.5), 67 (16.5), 57 (3.4), 55 (14.2).
14.59	5.7	154/$C_{10}H_{18}O$	cis-Sabinene hydrate	154 [M^+] (1.2), 136 (5.50, 121 (11.4), 111 (14.0), 93 (28.1), 71 (46.7), 58 (10.3), 55 (23.4), 43 (100).
15.26	Verytrace	154/ $C_{10}H_{18}O$	trans Pine hydrate	154 [M^+] (1.16), 136 (5.44), 111 (13.94), 93 (28.08), 81 (25.28), 71 (46.67), 71 (46.67), 55 (46.67), 43 (100).
15.48	Trace	154/ $C_{10}H_{18}O$	cis Pine	154 [M^+] (1.16), 136 (5.44), 111 (13.94), 93 (28.08), 81 (25.28), 71 (46.67), 71 (46.67), 55 (46.67), 43 (100).
16.20	Trace	154/$C_{10}H_{18}O$	Pine-ol	154 [M^+] (1.84), 136 (6.67), 121 (10.39), 93 (26.80), 69 (24.17), 55 (18.52), 43 (100).
16.77	Trace	150/$C_{14}H_{24}$	5-Tetradecane-3-yne	150 [M^+] (9.91), 125 (5.33), 91 (49.75), 79 (100), 67 (33.92), 41 (61.60).
17.87	17.6	154/$C_{10}H_{18}O$	Terpenol-4	154 [M^+] (5.3), 136 (6.7), 111 (26.7), 93 (37.9), 81 (9.9), 71 (100), 55 (25.5), 43 (18.20).
18.29	Trace	154/$C_{10}H_{18}O$	α-Terpineol	154 [M^+] (not appeared), 139 (3.37), 136 (19.07), 121 (23.28), 107 (3.41), 93 (39.26), 81 (28.37), 59 (100), 43 (52.12).
18.80	Very trace	154/$C_{10}H_{18}O$	Piperitol	154 [M^+] (3.27), 139 (19.52), 121 (5.61), 111 (6.64), 93 (27.13), 84 (100), 77 (16.81), 55 (27.39), 41 (47.63).

R_t.(min)	Rel. abund (%)	Mol. Wt.	Name of comp.	Molecular ions and their fragmentations m/z (%)
20.11	Trace	150 $C_{10}H_{14}O$	Carvone	150 [M^+] (4.29), 133 (6.40), 119 (3.48), 108 (20.97), 91 (15.30), 82 (100), 67 (9.99), 54 (66.26), 41 (38.16).
21.80	Trace	150/$C_{10}H_{14}O$	Carvacrol	150 [M^+] (4.29), 133 (6.40), 119 (3.48), 108 (20.97), 91 (15.30), 82 (100), 67 (9.99), 54 (66.26), 41 (38.16).
26.64	Trace	204/$C_{15}H_{24}$	trans Caryophyllene	204 [M^+] (1.5), 189 (5.0), 175 (2.5), 161 (9.9), 147 (9.8), 133 (31.9), 121 (11.1), 119 (15.8), 105 (26.0), 93 (47.6), 79 (43.2), 69 (54.8), 55 (27.6), 41 (100).
27.65	Trace	204/$C_{15}H_{24}$	β-Selinene	204 [M^+] (2.8), 189 (1.7), 161 (2.1), 147 (12.2), 136 (2.1), 121 (22.3), 107 (12.2), 93 (100), 80 (39.0), 67 (18.2), 53 (16.5), 41 (48.0).
28.96	Trace	204/$C_{15}H_{24}$	Bicyclo-germacrene	204 [M^+] (9.0), 189 (6.4), 161 (20.3), 147 (5.1), 136 (13.7), 121 (100), 107 (47. 4), 93 (85.4), 79 (59.0), 67 (34.3), 53 (33.3), 41 (95.2).
29.65	Trace	204/$C_{15}H_{24}$	δ-Cadinene	204 [M^+] (3.65), 189 (19.13), 161 (100), 147 (8.13), 134 (69.29), 119 (90.28), 105 (82.42), 91 (60.0), 81 (50.50), 65 (12.29), 55 (33.79), 41 (82.35).
31.57	2.1	220/$C_{15}H_{24}O$	(+) Spthulenol	205 [M^+] (15.7), 187 (8.0), 177 (3.6), 159 (13.7), 147 (11.3), 131 (9.2), 119 (21.0), 105 (21.5), 91 (33.1), 79 (25.0), 67 (20.8), 55 (20.8), 43 (100), 41 (65.5).
31.76	3.0	220/$C_{15}H_{24}O$	(-) Caryophyllene	205 [M^+] (1.5), 187 (1.5), 177 (2.2), 161 (4.5), 149 (5.9), 135 (4.9), 121 (12.1), 107 (17.9), 91 (34.0), 79 (54.5), 55 (35.0), 41 (100).
34.40	3.2	188/$C_{12}H_{12}O_2$	Butylidenephthalide	188 [M^+] (15.2), 173 (1.1), 159 (100), 146 (38.5), 131 (34.4), 115 (8.2), 103 (32.0), 91 (4.9), 77 (37.3), 55 (21.6), 41 (11.0).
36.36	10.5	190/$C_{12}H_{14}O_2$	trans Ligustilide	190 [M^+] (28.4), 175 (0.4), 161 (50.3), 148 (48.5), 133 (12.7), 120 (10.0), 105 (50.5), 91 (15.5), 77 (36.3), 55 (100), 41 (11.6).
36.60	22.8	190/$C_{12}H_{14}O_2$	cis Ligustilide	190 [M^+] (4.4), 175 (0.5), 161 (56.9), 148 (56.8), 133 (15.7), 105 (53.8), 91 (16.4), 78 (35.5), 55 (100), 51 (24.8), 41 (11.8).
37.64	Trace	190/$C_{12}H_{14}O_2$	Not identified	190 [M^+] (31.3), 175 (0.6), 161 (56.6), 148 (55.2), 133 (15.6), 120 (10.8), 105 (53.6), 91 (15.4), 78 (35.4), 55 (100), 41 (11.2).
37.92	Very trace	190/$C_{12}H_{14}O$	Not identified	190 [M^+] (24.43), 161 (44.66), 148 (43.48), 133 (10.61), 120 (9.48), 105 (45.55), 91 (15.29), 77 (35.29), 55 (26.44).
39.62	Very trace	278/$C_{16}H_{22}O_4$	Di-isobutyl benzene-2-dicaboxylate	278 [M^+] (not appeared), 223 (4.98), 205 (1.52), 167 (3.37), 149 (100), 132 (1.55), 121 (2.16), 104 (8.77), 93 (4.07), 76 (9.44), 57 (49.30), 41 (32.66).

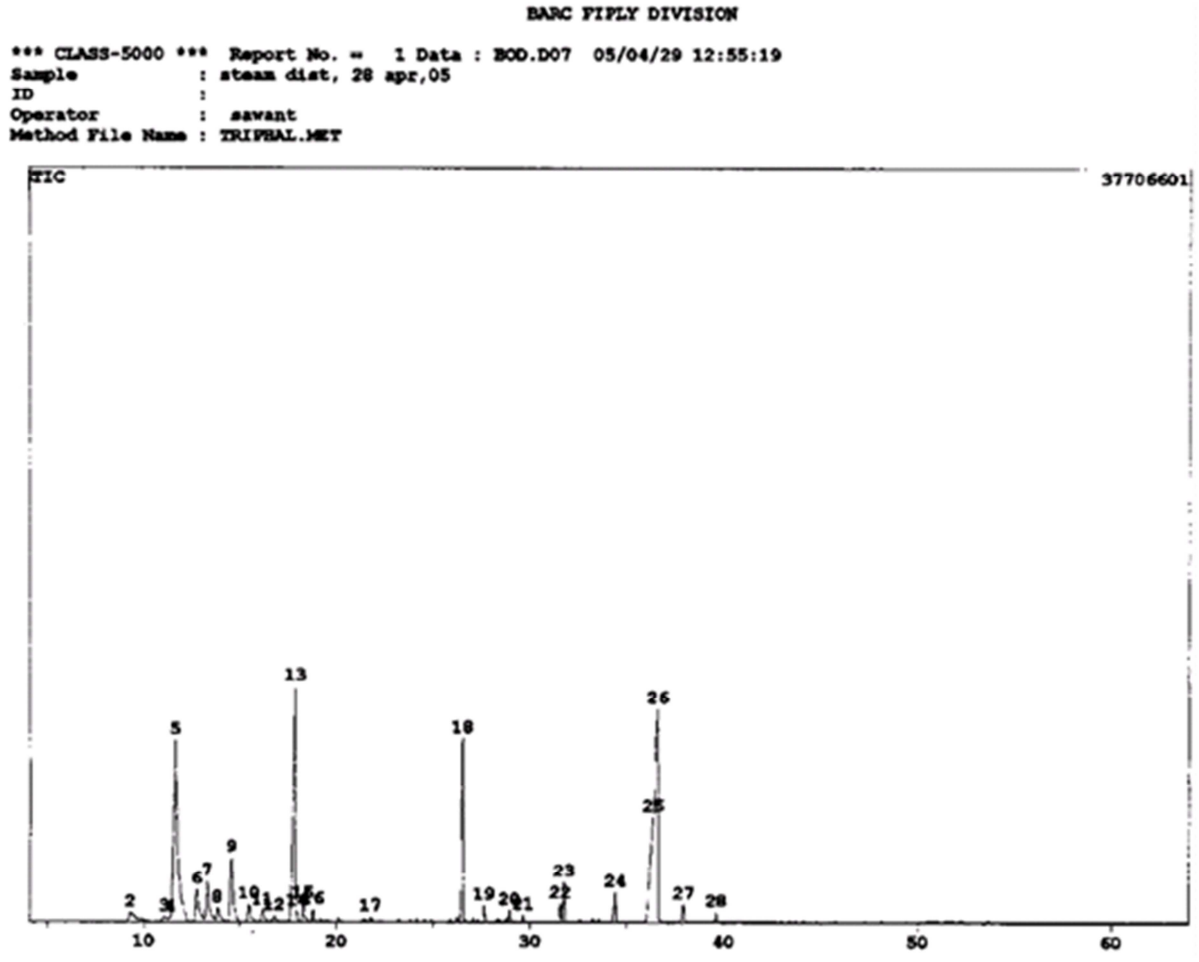

Figure 1. *GC profile of steam distillate of T. roxburghianum seeds.*

Table 2. *Report of GCMS analysis of steam distillate of T. roxburghianum seeds.*

Peak No	R. Time	I. Time	F. Time	Area	Height	A/H (sec)	MK	% Total	Name
1	4.061	3.508	4.117	684465988	35165654	19.464		53.67	
2	9.324	9.175	9.667	6215443	404488	15.366		0.49	
3	11.077	10.950	11.292	2845451	210100	13.549		0.22	
4	11.392	11.292	11.425	1282110	190581	6.727	V	0.10	
5	11.659	11.425	12.292	134605265	9024974	14.915	V	10.55	
6	12.767	12.617	13.158	19130411	1607768	11.899		1.50	
7	13.304	13.158	13.758	23571531	2006066	11.750	V	1.85	
8	13.850	13.758	14.325	7119410	662709	10.743	SV	0.56	
9	14.586	14.325	14.958	29261493	3177566	9.209		2.29	
10	15.476	15.383	15.858	7264288	838638	8.662	V	0.57	
11	16.182	16.092	16.517	4542624	557648	8.146	V	0.36	
12	16.770	16.517	17.075	2604468	266526	9.772		0.20	
13	17.866	17.542	17.933	90490309	11724552	7.718		7.10	
14	17967	17.933	18.083	1856849	477614	3.888	V	0.15	
15	18.286	18.200	18.533	6068658	852106	7.122	V	0.48	
16	18.800	18.717	19.000	2807157	577762	4.859		0.22	
17	21.784	21.658	21.875	1226022	247828	4.947	V	0.10	
18	26.536	26.342	26.592	47115567	9147846	5.150	V	3.69	
19	27.645	27.550	27.750	2988016	818322	3.651		0.23	
20	28.954	28.867	29.083	2147505	573382	3.745	V	0.17	
21	29.649	29.575	29.742	1143200	334889	3.414		0.09	
22	31.571	31.467	31.650	3713775	885378	4.195		0.29	
23	31.762	31.650	31.917	7911995	2002357	3.951	V	0.62	
24	34.398	34.225	34.483	7718918	1470984	5.247		0.61	
25	36.358	35.958	36.367	53861682	5187863	10.382		4.55	
26	36.601	36.367	36.767	117370843	10585704	11.088	V	9.20	
27	37921	37.758	38.000	4281125	861224	4.971		0.34	
28	39.602	39.508	39.683	1719511	489642	3.512		0.13	
Total				1275329608				100.00	

4. Conclusion

Based on the above experimental result, it has been found that the major constituents of steam distillate obtained from *T. roxburghianum* seed are 1-limonene (26.12%), terpenol (17.6%), *trans* ligustilide (10.5%) and *cis* ligustilide (22.8%) respectively along with other minor constituents as shown in Table 1, established by GCMS analysis using FID as detector and compare with authentic database available in GCMS library.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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