

Isolation and Identification of the Major Chemical Components of the Medicinal Plant *Trachyspermum copticum* (*Ammi copticum*) Grown in Fars Province of Iran

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Extract of the fruits of *Ammi copticum* plant (*Trachyspermum copticum*), grown wildly in the Fars province of Iran, in various solvents were obtained and analyzed. GC-MS analysis of the extract and of the essential oil obtained from the fruit of the plant showed 138 components. Thymol (65 %) and linoleic acid (13.5 %) were isolated and characterized as the major chemical constituents.

Key Words: *Ammi copticum*, *Trachyspermum copticum*, Thymol, 5-Methyl-2-(1-methylethyl)phenol, Linoleic acid, Essential oil.

INTRODUCTION

Fruit of *Ammi copticum* plant (*Trachyspermum copticum*) has been used in traditional medicine since ancient times up to now. This plant is wildly grown in the Fars province of Iran. The plant is a member of Umbelliferae family, belongs to the class of Dicotyledones and a subclass of Dialypetales. The fruit of the plant is carminative, anti nausea and vermicide. It is also diuretic and has been used for the treatment of gastric disorders and acute poisonous cases. Regarding the medicinally importance of this plant and its widespread occurrence in the Fars province of Iran, the following objectives were investigated in this research: (1) isolation and purification of the major chemical compounds possibly found in the extracts of the plant (in various polar and non-polar solvents), (2) identification and structural elucidation of the isolated major chemical compound(s) by using various spectroscopic techniques and (3) composition analysis of the chemical compounds found in the essential oil obtained from the fruit of the *Ammi copticum* plant by using GC-MS technique.

EXPERIMENTAL

The fruits and the aerial parts of the wild-growing *Ammi copticum* plant (*Trachyspermum copticum*) were collected from the surrounding

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mountains of Fars province, of Iran. The plant was identified as *Trachyspermum copticum* by the Herbarium Department and Faculty of Agriculture of Shahid Chamran University, Ahwaz, Iran.

Maceration of the powdered fruit in various solvents such as water, methanol, chloroform and others were carried out at room temperature for 48 h. Soxhlet extraction of the powdered fruit (100 g) by various solvents (600 mL) were also carried out. The best solvent was found to be toluene. In each method, a pale yellow oil was obtained. 6.4 g of a yellow oil was obtained from the soxhlet extraction method. The progress of the extraction methods were monitored by TLC and Infrared spectra. The whole fruits of the plant were powdered (100 g) and the volatile fraction was isolated by hydrodistillation, a liquid oil (3.8 g) was obtained. The oil was dried over anhydrous sodium sulphate and kept in refrigerator (4°C). The oil had an $n_D = 1.4597$ at 27.3°C.

Analysis

The crude oil (extract and the essential oil) was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). GC analysis of the oil was conducted using a Thermoquest-Finnigan instrument equipped with a DB-1 fused silica column (30 m \times 0.32 mm i.d., film thickness 0.25 μ m). Nitrogen was used as the carrier gas at the constant flow of 1.1 mL/min. The oven temperature was held at 50°C for 1 min, then, programmed to 265°C at a rate of 4°C/min and then held for 10 min. The injector and detector (FID) temperatures were kept at 280°C.

GC-MS analysis was carried out on a Thermoquest-Finnigan. Trace GC-MS instrument equipped with a DB-1 fused silica column (60 m \times 0.25 mm i.d., film thickness 0.25 μ m). The oven temperature was raised from 50° to 265°C at a rate of 5°C/min, then held at 265°C for 10 min; transfer line temperature 265°C. Helium was used as the carrier gas at a flow rate of 1.5 mL/min; split ratio, 1/50. The quadrupole mass spectrometer was scanned over the 45-500 amu with an ionizing voltage of 70 eV and an ionization current of 150 μ A. The constituents of the volatile oil were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C₆-C₂₄) and the oil on a DB-1 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds or with those reported in the literature^{1,2} as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature. Quantitative data was obtained from FID area percentages without the use of correction factors.

RESULTS AND DISCUSSION

TLC and column chromatography (on silica gel and ether as the mobile phase) on both, the extracts and the essential oil resulted in the isolation of pure thymol as the major chemical compound. From ¹H NMR, ¹³C NMR, IR, UV and mass spectra of the fraction, its chemical structure was elucidated and confirmed. GC-MS analysis of the extract resulted in the separation of 139 components (Table-1). Similar results were obtained from the essential oil of the powdered fruits of the plant as well. GC-MS of the extract showed (Table-2) 9 major components; thymol [5-methyl-2-(1-methylethyl)phenol] (I) (65 %) and linoleic acid (II) (13.5 %), were the major constituents. The chemical structures of components (1-8) are given in Fig. 1.

TABLE-1
GC-MS ANALYSIS OF THE EXTRACT OBTAINED FROM THE FRUIT OF
AMMI COPTICUM PLANT OF FARS PROVINCE

Peak List No Name 01.Raw RT : 0.00-116.47
Number of detected peaks: 139

Apex RT	Start RT	End RT	Area	Area (%)	Height	Height (%)
3.21	3.08	4.92	72128246	1.29	1454620.00	0.69
6.21	6.16	6.42	498378.3	0.01	59719.84	0.03
7.28	7.19	7.70	2989340	0.05	251900.00	0.12
7.81	7.75	7.99	700004.1	0.01	99889.15	0.05
9.51	9.43	9.76	1977371	0.04	247344.10	0.12
9.83	9.76	10.22	3508803	0.06	341351.30	0.16
11.35	11.28	11.57	1501586	0.03	266428.20	0.13
11.77	11.60	12.14	903882.7	0.02	53091.66	0.03
13.27	13.12	13.82	16306730	0.29	2626149.00	1.24
14.37	13.82	14.64	4260626	0.08	208132.80	0.10
15.23	15.00	15.52	632719.8	0.01	55859.77	0.03
16.23	16.12	16.36	409693.3	0.01	58105.13	0.03
16.57	16.50	16.69	385313.9	0.01	51271.57	0.02
16.76	16.73	16.97	486513.3	0.01	54114.91	0.03
17.08	16.97	17.47	1848890	0.03	126858.00	0.06
17.80	17.47	18.07	3574109	0.06	199622.70	0.09
18.46	18.37	18.57	589746	0.01	88654.41	0.04
18.79	18.68	19.06	3364650	0.06	439623.50	0.21
19.22	19.14	19.33	357638.8	0.01	55659.54	0.03
20.75	20.52	21.11	4428480	0.08	458123.10	0.22
21.81	21.63	22.30	16872466	0.30	1524335.00	0.72
22.52	22.42	22.64	768442	0.01	102587.20	0.05
22.68	22.64	22.86	489510.8	0.01	61089.96	0.03
22.96	22.86	23.06	573715.9	0.01	71580.25	0.03
24.43	23.32	24.60	3.6E+09	64.56	97604653.00	46.23
24.74	24.70	24.80	2098978	0.04	804471.60	0.38
25.72	25.64	25.92	2909270	0.05	586585.00	0.28
27.90	27.82	28.18	1253176	0.02	147704.60	0.07
28.40	28.18	28.60	985436.6	0.02	110491.90	0.05
29.17	29.07	29.30	1552045	0.03	300291.60	0.14

Apex RT	Start RT	End RT	Area	Area (%)	Height	Height (%)
29.65	29.55	29.80	600629.5	0.01	108184.00	0.05
30.79	30.57	30.86	394944.2	0.01	45753.94	0.02
31.01	30.89	32.09	53483331.0	0.96	5554927.00	2.63
32.36	32.23	32.56	6327685.0	0.11	1093117.00	0.52
32.81	32.71	32.94	2850322.0	0.05	524221.70	0.25
33.19	33.07	33.34	515726.4	0.01	78635.23	0.04
34.30	34.13	34.61	2689366.0	0.05	254758.80	0.12
35.66	35.58	35.76	440695.7	0.01	90126.77	0.04
36.34	36.22	37.56	24943217.0	0.45	2222477.00	1.05
37.59	37.56	37.66	237734.6	0.00	55487.42	0.03
37.75	37.66	37.89	674427.3	0.01	102275.40	0.05
38.94	38.83	39.17	1883815.0	0.03	282638.90	0.13
40.27	40.22	40.34	207640.8	0.00	52682.06	0.02
40.89	40.74	41.50	1871093.0	0.03	124366.60	0.06
42.12	42.04	42.25	546212.6	0.01	95810.82	0.05
42.31	42.29	42.50	334875.9	0.01	39058.55	0.02
43.52	43.37	44.16	11084761.0	0.20	1114610.00	0.53
44.47	44.38	44.58	313997.0	0.01	47969.18	0.02
44.82	44.72	45.11	3209424.0	0.06	335127.80	0.16
45.32	45.11	45.90	4234181.0	0.08	165881.10	0.08
46.30	45.90	46.79	3117672.0	0.06	163812.90	0.08
47.86	47.65	48.30	12162238.0	0.22	1691888.00	0.80
48.79	48.64	49.22	5212360.0	0.09	547156.40	0.26
49.62	49.22	49.84	25286132.0	0.45	4138384.00	196.00
50.33	49.84	50.60	15969294.0	0.29	2333137.00	1.10
51.09	50.91	51.24	2208306.0	0.04	358649.90	0.17
51.34	51.27	51.52	1237634.0	0.02	179210.90	0.08
52.05	51.99	52.10	183744.7	0.00	47268.41	0.02
52.65	52.10	52.82	4555998.0	0.08	310705.20	0.15
53.50	53.00	53.85	89934716.0	1.61	4838553.00	2.29
53.96	53.85	54.40	21474895.0	0.38	1565531.00	0.74
54.61	54.51	54.71	1464545.0	0.03	290918.30	0.14
55.19	55.01	55.58	4567446.0	0.08	253096.80	0.12
55.76	55.58	55.88	3904474.0	0.07	363046.30	0.17
56.54	56.07	57.35	12566563.0	0.23	522562.60	0.25
57.81	57.35	58.21	18955644.0	0.34	1533753.00	0.73
58.89	58.53	59.12	11650534.0	0.21	844273.10	0.40
60.72	59.63	61.35	7.57E+08	13.56	22061847.00	10.45
61.55	61.45	61.67	1436955.0	0.03	218848.40	0.10
61.78	61.76	61.84	318798.8	0.01	126275.40	0.06
61.85	61.84	62.04	1023428.0	0.02	124306.10	0.06
62.16	62.04	62.27	1365119.0	0.02	206192.40	0.10
62.39	62.33	62.51	992418.9	0.02	191893.50	0.09
62.83	62.74	62.86	496167.3	0.01	104111.50	0.05
62.90	62.86	63.04	597816.7	0.01	106413.50	0.05
63.53	63.42	63.75	1772745.0	0.03	213992.50	0.10
64.27	64.15	64.41	1270825.0	0.02	140439.00	0.07

Apex RT	Start RT	End RT	Area	Area (%)	Height	Height (%)
64.56	64.44	64.73	5165223	0.09	733733.50	0.35
64.93	64.73	65.35	3920284	0.07	209544.10	0.10
65.61	65.48	66.03	7910170	0.14	743273.50	0.35
66.21	66.03	66.62	7261015	0.13	660347.90	0.31
66.73	66.62	67.02	3029236	0.05	227196.70	0.11
67.18	67.02	67.30	733583.6	0.01	65387.05	0.03
67.78	67.63	67.93	3444909	0.06	486739.70	0.23
68.19	68.11	68.28	392354.9	0.01	78627.09	0.04
68.67	68.30	69.24	8480357	0.15	421758.00	0.20
69.49	69.35	69.80	6370995	0.11	631408.10	0.30
70.09	69.90	70.70	22409524	0.40	1077714.00	0.51
70.86	70.70	71.14	10398999	0.19	1401822.00	0.66
71.87	71.76	72.12	1374929	0.02	137313.70	0.07
72.33	72.22	72.81	3555011	0.06	398282.10	0.19
73.14	72.87	73.33	3568227	0.06	406301.50	0.19
73.83	73.66	74.01	4032777	0.07	556916.70	0.26
74.80	74.74	74.89	310033.3	0.01	58824.74	0.03
75.33	75.09	75.80	10457969	0.19	531545.70	0.25
75.95	75.80	76.56	6020057	0.11	241010.70	0.11
76.73	76.56	77.28	18081842	0.32	2218328.00	1.05
77.66	77.51	77.82	2730243	0.05	285878.30	0.14
78.19	77.82	78.58	14656514	0.26	1348068.00	0.64
78.72	78.58	78.85	1840762	0.03	237097.30	0.11
79.06	78.97	79.14	392655.7	0.01	64512.66	0.03
79.48	79.23	79.81	9287668	0.17	993117.20	0.47
80.23	79.81	80.55	27354677	0.49	1757490.00	0.83
80.97	80.55	81.26	6414270	0.11	260485.50	0.12
81.42	81.26	81.59	2228520	0.04	218529.70	0.10
81.78	81.68	81.97	1187651	0.02	127641.60	0.06
82.19	81.97	82.51	56813470	1.02	7939133.00	3.76
82.72	82.58	82.81	360178.6	0.01	49574.35	0.02
83.10	82.84	83.52	6139986	0.11	396356.60	0.19
83.64	83.55	83.83	1833931	0.03	241841.70	0.11
84.06	83.83	84.28	3557828	0.06	242378.30	0.11
84.75	84.37	85.01	10775847	0.19	700839.40	0.33
85.15	85.06	85.39	1476535	0.03	122722.80	0.06
85.80	85.46	86.10	4120006	0.07	205306.80	0.10
86.31	86.10	86.55	3802853	0.07	260271.50	0.12
86.72	86.55	86.97	1945490	0.03	130287.70	0.06
87.31	87.12	87.60	15187627	0.27	1692928.00	0.80
87.65	87.60	87.81	418888.3	0.01	46959.21	0.02
88.22	87.81	88.71	9205896	0.16	354701.40	0.17
88.85	88.71	89.17	3024414	0.05	188391.00	0.09
89.50	89.17	90.06	15265375	0.27	1126384.00	0.53
90.30	90.14	90.59	2366374	0.04	166581.90	0.08
90.90	90.59	91.60	6785294	0.12	254110.40	0.12
91.94	91.73	92.97	47731082	0.86	18874490.00	0.89

Apex RT	Start RT	End RT	Area	Area (%)	Height	Height (%)
94.06	93.66	94.72	53542765	0.96	1559846.00	0.74
94.98	94.72	95.86	22517243	0.40	702855.70	0.33
96.37	96.07	96.73	7397443	0.13	326268.50	0.15
97.03	96.73	97.92	73452773	1.32	2900601.00	1.37
98.34	98.20	98.44	325794	0.01	38071.53	0.02
99.48	99.24	100.65	10235948	0.18	326166.80	0.15
102.20	101.57	102.62	1.49E+08	2.67	7726189.00	3.66
103.82	103.49	104.15	2696112	0.05	135088.40	0.06
105.53	105.12	105.82	908559.9	0.02	50158.20	0.02
106.70	106.20	106.89	2242685	0.04	86452.09	0.04
107.43	106.89	107.78	4229268	0.08	157612.70	0.07
108.17	108.09	108.48	702074.5	0.01	55171.79	0.03
113.33	113.03	113.35	484982.9	0.01	54282.51	0.03
113.47	113.35	113.58	562891.5	0.01	48936.25	0.02
113.65	113.58	114.13	623593.2	0.01	30894.64	0.01

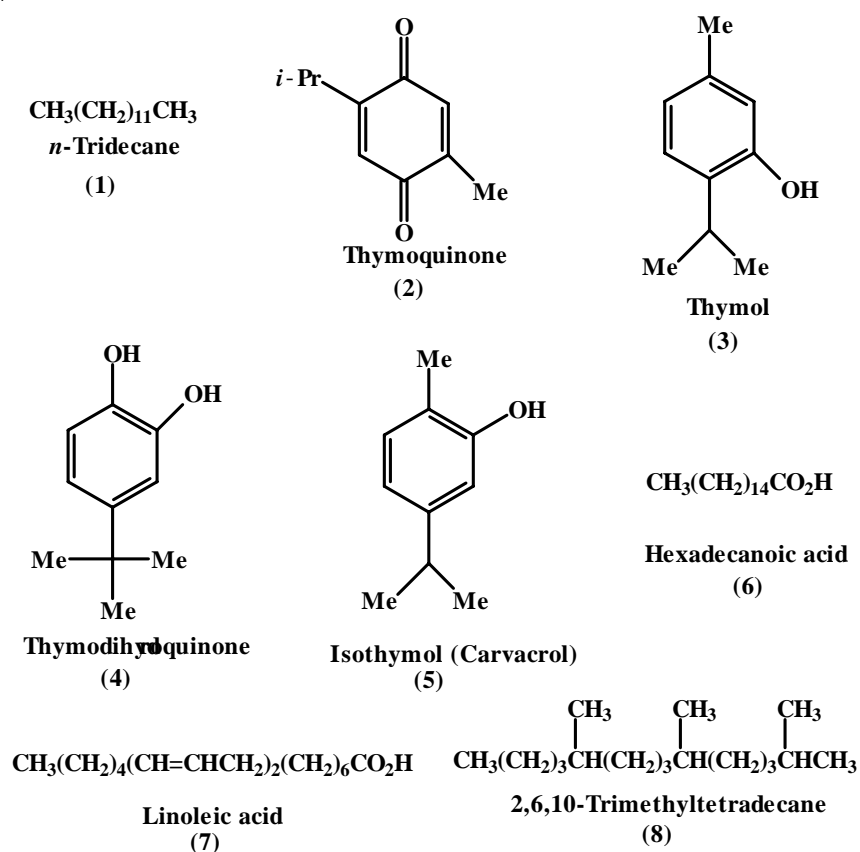


Fig. 1. Chemical structures of the major components found in the extract of the Fruit of *Ammi copticum* plant of Fars Province

TABLE-2
THE 9 MAJOR CHEMICAL COMPOUNDS IDENTIFIED IN THE
EXTRACT OBTAINED FROM THE FRUIT OF *AMMI COPTICUM* PLANT
OF FARS PROVINCE

No.	Apex R.T. (min)	Area (%)	Height (%)	Name of the compound	m.w.
1	13.27	0.29	1.24	<i>n</i> -Tridecane	180
2	21.81	0.30	0.72	Thymoquinone	164
3	24.43	64.59	46.23	Thymol	150
4	31.01	0.96	2.63	Thymol dihydroquinone	166
5	49.62	0.45	1.96	Isothymol (Carvacrol)	150
6	53.5	9.69	2.29	Hexadecanoic acid	256
7	60.72	13.56	10.45	Linoleic acid	280
8	76.70	0.32	1.05	2,6,10-Trimethyl tetradecane	240
9	102.2	2.67	3.66	LUP-20(2q)-en-3-ol, acetate	486

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