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RESEARCH PAPER

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A comparative study on the chemical composition of oil obtained from whole seeds and crushed seeds of *Nigella sativa* L. from India

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ABSTRACT

Seeds of Nigella sativa L. have rich medicinal values and are frequently used in folk medicine in the Middle East and some Asian countries including India for the promotion of good health and treatment of many common and chronic diseases. Many valuable active molecules in the essential oil can be frequently attributed to these pharmacological properties. The essential oil obtained by the solvent extraction method of whole seeds and crushed seeds of Nigella sativa was investigated by Gas Chromatography and Gas chromatography Mass Spectrometry techniques indicating the presence of total forty three components in both, accounting for 95% of oil. In it some major components like thymoquinone, p-cymene, palmitic acid, oleic acid and lenoleic acids are inter- convertible, while one increased in crushed seed the other decreased in whole seed and it is vice-versa. Here for the first time, comparative study on the extraction and quality of the essential oil from the whole and crushed seeds of N. sativa is investigated.

KEYWORDS: Thymoquinone, Ranunculaceae, Solvent extraction, GC and GCMS.

INTRODUCTION

Nigella sativa L. belonging to the family Ranunculaceae had originated in Turkey and Italy and is now commonly grown in Europe, Middle East and Western Asia. It is also being cultivated throughout India.

Plant is an annual herb; the height of the plant is approximately half a meter. The leaves are dark green, cauline, dissected (coriander like), Flowers have five petals, white with blue veins and the plant appears between June- September. Seeds are small approximately 1 to 2mm long, triangular in shape, black in colour with rough surface and it possess a severe pungent smell. The fixed oil of seeds contain unsaturated fatty acids mainly linoleic acid, linolenic acid, oleic acid, palmitolic acid (Babayan *et al.,* 1978; Gad *et al.,* 1986) and saturated fatty acids are palmitic acid, stearic acid and myristic acid (Babayan *et al.,* 1978; Gad *et al.,* 1986; Menounos *et al.,* 1963; Nagi *et al.,*1999) are also present in the oil.

In the seed oil thymoquinone was identified as the main component besides p-cymene, α-pinene, dithymoquinone, thymohydroquinone and nigellone, other terpene derivatives were found only in traces; carvacrol, carvone, limonene, terpineol-4, citronellol (El-Dakhakhany, 1963; El-Fatatry,1975; Enomoto *et al.,* 2001; Ghosheh *et al.,* 1999) furthermore traces of alkaloids i.e. nigellicine, nigellidine, nigellimine-N-oxide and coumarin (Ata-ur-Rehman & Malik. 1995; Ata-ur-Rehman *et al.,*1985a&b; Drozed *et al.,* 1970; El- Zawahry,1964) are reported.

Due to the presence of these constituents seeds of *N. sativa* has richmedicinal properties and are frequently used in folk medicine in the middle East and some Asian countries including India for the promotion of good health and treatment of many diseases including fever, common cold, headache, asthma, rheumatic diseases, various microbial infections and to expel worms from the intestine because it include immune stimulation (El-Kadi & Kandil, 1986; Kumara & Huat, 2001), anti-inflammatory (Houghton *et al.,* 1995), haemostatic (Al-Jishi, 2000), anti-cancer (Salomi et al., 1991), anti-microbial (El-Fatatry,1975; Topozada *et al.,* 1965), anti-parasitic (Akhtar & Riffat, 1991), anti-oxidant (Badary *et al.,* 2000; Nair *et al.,* 1991) and hypoglycemic (Al-Awadi & Gumma,1987; Bamosa *et al.,*1997) effects etc. *Nigella* is considered a BRM (Biological Response Modifiers) because the studies show extract from the seeds are toxic to cancer cells in mice and prevents the toxic effect of anticancer drug cisplatin (Nair *et al.,* 1991). *Nigella* also stimulates the immune system, as shown in an experiment conducted with human lymphocytes. Cells treated with *Nigella* seeds protein produced greater amount of cytokines, specifically interleukin-1-beta and tumor necrosis factor alpha (Kumara & Huat, 2001).

Seed oil of *N. sativa* and pure thymoquinone are inhibitor of eicosanoid generation and membrane lipid peroxidation both inhibited the cyclo- oxygenase and 5-lipoxygenase pathways of arachidonate metabolism in rat (Houghton *et al.,* 1995). However there is no report on comparative chemical analysis of oil from the whole and crushed seeds of *N. sativa*. This paper report compositional change in oil obtained through solvent extraction from the crushed and whole seeds of *N. sativa.*

MATERIAL AND METHODS

Plant Material

The seeds of *N. sativa* L. were collected in February 2006 from direct cultivars near the country side of Lucknow, India after the flowering stage.

Extraction and Isolation of Oil

The oil was obtained by solvent extraction method. The seeds, divided into two parts (50gm each), I^{st} is (WS) whole seed and II^{nd} is (CS) crushed seed. Then both type of seeds kept into conical flask and filled with 150ml solvent (Dichloromethane), the flask close with cork and left for a week. After that the seeds filtered through Whatman filter paper. The total filtrate is found about 100ml of each, the filtrate kept open on room temperature to evaporate the solvent. After evaporation the remaining part is 5ml of each left. The comparative compositions of area percentage of constituents through gas chromatography in both the seed oils of *N. sativa* have been shown in Table- 1.

Gas Chromatography

The Gas chromatography analysis was performed on a Perkin Elmer *Auto System XL*, with FID attachment and an Equity -5 column (5% Phenyl ethyl glycol 95% dimethyl polysiloxane), fused silica capillary column (60m x 0.32mm) and 0.25µm film thickness. Injector temperature 220^0C and detector temperature 350^0C oven temperature was programmed from 70^0C to 180^0C at rate of 3^0C/minute, with initial hold of 2 minute and from 180^0C to 350^0C at the rate of 10^0C/minute with final hold of 5 minute. Hydrogen is used as carrier gas with column head pressure 10 psi, split ratio was 1:30. Total Chrom software was used for the processing the data from FID, and Control Chrom for Kovat Indices.

GC-MS data were obtained at 70eV on Perkin Elmer Mass spectrometer using a PE-5 column (50m x 0.32mm) and 0.25 mm film thickness. The carrier gas was helium and the same temperature programming was done.

The constituents were identified by comparison of their Mass spectra with those in the computer library MS data (NIST and WILEY) or with authentic compounds and confirmed by comparing their retention indices (RI) of the peaks on a PE-5 column determined with reference to a saturated alkane mixture C_8-C_{23} with those of authentic compounds data reported in literature.

RESULTS AND DISCUSSION

The essential oils obtained from the whole seeds (WS) of *Nigella sativa* through solvent (dichloromethane) extraction have great variation with the essential oils obtained from crushed seeds (CS) of *Nigella sativa* the analytical results (figure-1&2) of both the oils are given in table-1. The result shows very typical effect of crushing of the seeds on the composition of the oils. About six constituents show enormous effect namely p-cymene (WS=2.0%, CS=10.6%), menthol (WS=nil, CS=2.9%), thymoquinone (WS=8.9%, CS=15.9%), palmitic acid (WS=2.3%, CS=6.8%), linoleic acid (WS=28.5%,

CS=41.1%), oleic acid (WS=16.4%, CS=3.5%). Beside these other variations are absence of certain constituents in WS while appearing in traces in CS and vice-versa.
It is interesting to note that the total amount of linoleic acid and oleic acid is nearly same (44.9 in WS and 44.6 in CS) in both oils but individually they are very different as is evident from Table-1, palmitic acid is also nearly 3 times of WS (2.3%) in CS (6.8%).
In the light of the said work, it can be inferred that the difference in whole and crushed seeds oils is due to enzyme (lipase) activity in the seeds (Kamal-Eldin & Appelqvist, 1995; Sahasrabudhe, 1982; Tookey & Wolf, 1964). Although the present study provides new information about the chemical composition and the effect of crushing on it much still remains to be studied at biochemical level.

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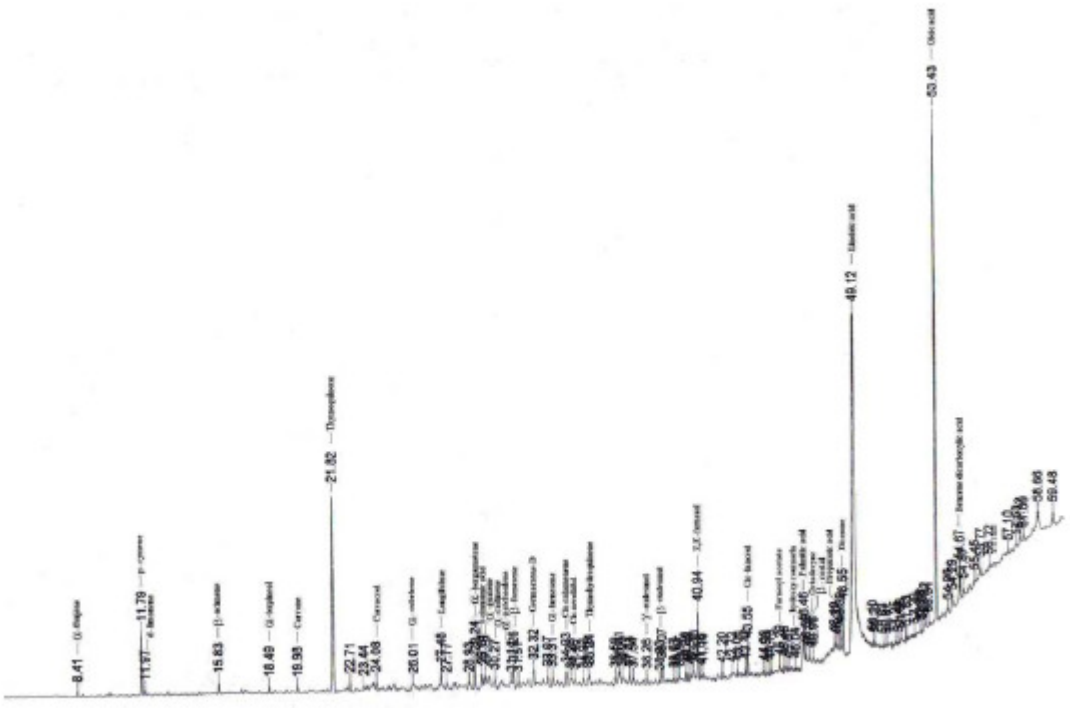

Figure 1. Chromatogram of oil from whole seeds of *Nigella sativa*

Table 1. Comparative composition of seed oil of *Nigella sativa* L.

S.No.	Chemical constituents	K.I	Oil of W.S (area %)	Oil of C.S (area%)	Method of Identification
1.	α-thujene	924.5	0.15	2.69	KI,MS
2.	α-pinene	932.6	x	0.63	KI,MS
3.	sabinene	971.5	x	0.31	KI,MS
4.	β-pinene	977.0	x	0.71	KI,MS
5.	p-cymene	1022.8	2.04	10.69	KI,MS
6.	limomene	1026.9	0.16	0.51	KI,MS
7.	γ-terpinene	1055.9	x	0.12	KI,MS
8.	α-thujone	1095.8	x	0.20	KI,MS
9.	β-ocimene	1118.9	0.44	1.23	KI,MS
10.	isomenthone	1153.7	x	0.22	KI,MS
11.	neo-menthol	1164.7	x	0.24	KI,MS
12.	menthol	1172.7	x	2.97	KI,MS
13.	α-terpineol	1178.3	0.25	0.22	KI,MS
14.	d-citronellol	1204.2	x	0.12	KI,MS
15.	carvone	1242	0.22	x	KI,MS
16.	thymoquinone	1251.4	8.95	15.90	KI,MS
17.	carvacrol	1299.0	0.31	0.54	KI,MS
18.	α-cubebene	1351	0.27	x	KI,MS
19.	citronelly acetate	1355.4	x	0.28	KI,MS
20.	longifolene	1371.6	0.79	0.06	KI,MS
21.	α-bergamotene	1412.2	1.25	1.07	KI,MS
22.	t-cinnamic acid	1422	0.65	x	KI,MS
23.	α-guaiene	1424.2	0.40	0.12	KI,MS
24.	(E),β-farnesene	1457.9	0.78	0.09	KI,MS
25.	germacrene-D	1482.5	1.10	0.05	KI,MS
26.	α-farnesene	1508	0.50	x	KI,MS
27.	cis-calamenene	1521	0.81	x	KI,MS
28.	cis-nerolidol	1534	0.37	x	KI,MS
29.	thymohydroquinone	1551.2	0.49	0.22	KI,MS
30.	γ-eudesmol	1630	0.35	x	KI,MS
31.	β-eudesmol	1649	0.81	x	KI,MS
32.	Z,E-farnesol	1690.9	3.05	0.36	KI,MS
33.	cis-lanceol	1760.7	1.05	0.13	KI,MS
34.	Z,E-farnesyl acetate	1818	0.31	x	KI,MS
35.	7-hydroxy coumarin	1829.7	0.10	0.15	KI,MS
36.	palmitic acid	1840.6	2.31	6.83	KI,MS
37.	octadecyne	1843.3	0.77	0.52	KI,MS
38.	β-costal	1849.1	0.69	0.35	KI,MS
39.	propanoic acid	1893.0	0.91	0.36	KI,MS
40.	eicosane	1897.5	1.26	2.53	KI,MS
41.	linoleic acid	1914.0	28.58	41.09	KI,MS
42.	oleic acid	2039.3	16.45	3.52	KI,MS
43.	benzene-dicarboxylic acid	2078.1	1.26	1.65	KI,MS

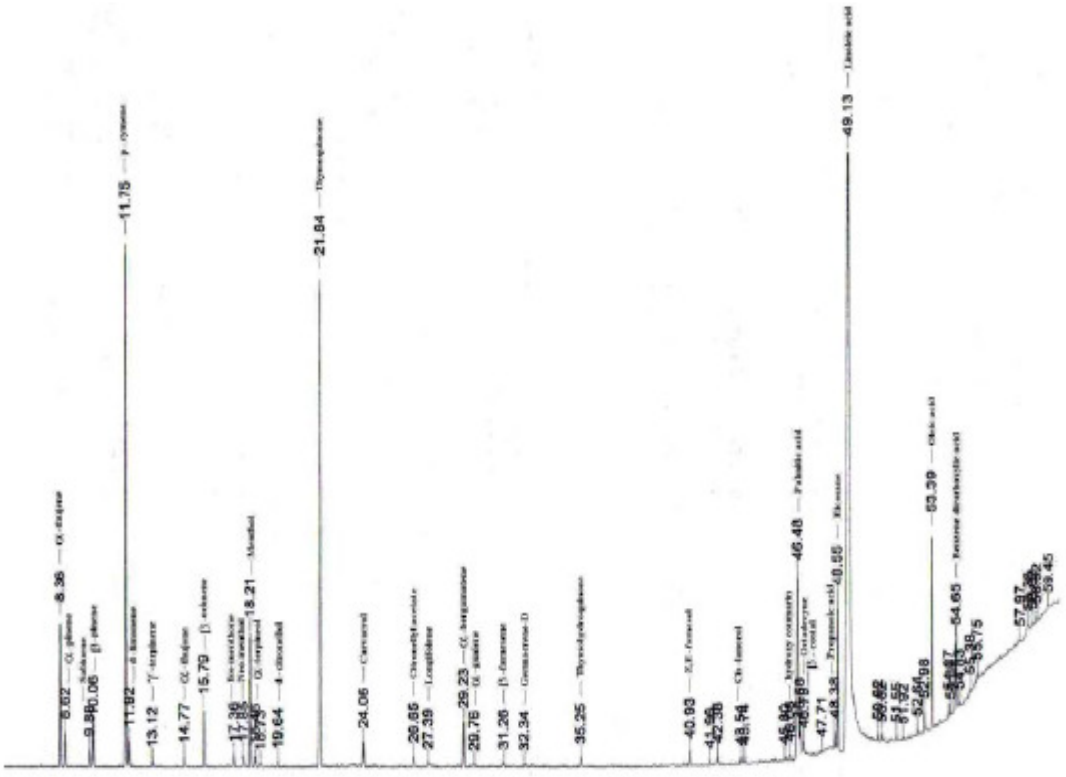

Figure 2. Chromatogram of oil from crushed seeds of *Nigella sativa*

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