

ESSENTIAL OIL AND LIPIDS OF NIGELLA SATIVA SEED AND THEIR BIOLOGICAL ACTIVITY

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ABSTRACT

The essential oil as well as the expressed oil of Nigella sativa L. seeds, were subjected to qualitative and quantitative studies employing different chromatographic techniques. Thymoquinone (ca 30%), p-cymene (ca 38%) and α -pinene (ca 14%) constituted the main make up of the essential oil. GLC revealed that the lipid portion of the expressed oil is built-up mostly of stearic (ca 47%) and arachidic (ca 32%) acids beside lesser amounts of palmitic (ca 7%) and myristic (ca 4%) acids. α -amyrin, β -sitosterol and oleanolic acid were isolated from the unsaponifiable matter of the lipid portion. The expressed oil showed significant antimicrobial and antifungal activities. It could normalized the enzymatic disturbance in the liver tissue, previously produced by exposure to ionizing radiation. This radioprotective effect was supported by the morphological changes of liver tissues. The intestine showed minimal changes and no evidence of protection was observed for the kidneys.

INTRODUCTION

The seeds of Nigella sativa L. are known in Arabic under the names : Habbah Sawda (black corn) (1) or Habbet El-Baraka (corn of blessing) (2). The Arabian authors (3-5) reported that the seeds are useful mainly in headache, respiratory appression, asthma and expelling the urinary calculus, as well as lactagogue, emmenagogue (6) and diuretic.

In Egyptian folk medicine (7) the seeds are used as carminative and are added as a flavouring agent to bread, while its expressed oil is used in asthma, respiratory appression and cough.

Reviewing the available literature, several authors (8-13) had investigated fixed and volatile oil to isolate and identify the active constituents which are of value in the treatment of asthma. The isolation of thymoquinone from the

essential oil of the seeds was ascribed to El-Dakhakhny (8) and thymohydroquinone to El-Alfy (10).

The present work was carried out to study the essential oil prepared by steam distillation of either the seeds or the expressed oil using TLC, TAS, TASOMAT and GLC methods. Moreover, screening of the antimicrobial and antifungal activities of both volatile and expressed oil were performed.

The work also was chiefly centered on evaluating the role of unsaponifiable and saponifiable moieties of the fixed oil in protecting the liver, kidney and intestine of mice and in promoting a recovery after a single lethal dose of body irradiation.

EXPERIMENTAL

Plant Material :

Nigella sativa L. seeds were

obtained from plants cultivated in the Experimental Station of Medicinal Plants, Faculty of Pharmacy Cairo University in June 1992. A herbarium of the plant (voucher specimen) is kept in the Museum of Medicinal Plants, Experimental Station, Faculty of Pharmacy, Cairo University.

Microorganisms and fungi used for screening :

Staphylococcus aureus, Micrococcus spp., Streptococcus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Candida albicans, Penicillium and Aspergillus spp. were kindly provided by the Microbiology Department, Faculty of Pharmacy, Cairo University.

Animals :

Male swiss albino mice weighing 20 g each, obtained from the breeding unit of the Cancer Biology Dept., National Cancer Institute, were used in these experiments. They were kept on a standard cube diet and free water supply. The animals were divided into four groups of mice each as follows :

- 1- Control (untreated) group.
- 2- Irradiated group.
- 3- N. sativa oil treated group.
- 4- N. sativa oil treated irradiated group.

Treatment :

Nigella sativa L. oil was administered, mixed with diet (10%) three days pre-irradiation. This mixed diet as is continued for three days post-irradiation. Irradiation had been performed at a dose level of 8G from ¹³⁷Cs g-radiation. Facility was provided by the National Center for Radiation Research and Technology, Cairo, Egypt.

Authentic :

- 1- Volatile oils were kindly supplied by the Pharmacognosy and Analytical Phytochemistry Institute, Saarlands University, Germany.
- 2- α - and β -amyrin, ursolic, oleanolic acids and β -sitosterol were kindly

provided from E. Merck, Darmstadt, Germany.

Apparatus :

Gas Chromatograph, Pye Unicam, Series 104, Model 64 with FID; TAS oven (Stahl), F. Desaga, Heidelberg, Germany; TASOMAT (Stahl), F. Desaga, Heidelberg, Germany; IR Spectrophotometer, Model 257, Perkin Elmer, Germany and Koffler's heating stage microscope, F. Leitz, Germany.

Percentage yield of volatile oil prepared by different methods :

- 1- 100 g of Nigella sativa L. seeds were subjected to steam distillation using Karlsruher Apparatus (14). The yield was found to be 0.72% w/w (calculated on dry weight). The oil was freed from solvents and directly used for further examination.
- 2- The percentage of volatile oil prepared by volumetric method was found to be 0.4% v/w (calculated on dry weight).
- 3- Four kg of Nigella sativa L. seeds were warmed at a temperature not exceeding 40°C, expressed by hydrolic pressure and the expressed oil (500 g) was subjected to steam distillation. The percentage of volatile oil was found to be 1.1% v/w of the expressed oil.

Physico-chemical constants of steam distilled volatile oil :

The oil has a yellow colour, changes to red on storage; it has an aromatic odour and taste;

refractive index	: 1.4619 ;
specific gravity	: 0.84 ;
acid value	: 0.1% ;
ester value	: 3.6% ;
carbonyl content	: 21%.

TAS and TASOMAT Study :

In the TAS method (15), the powdered

drug (in a glass patron) is inserted in a heating block of a known temperature. The sublimate or its degradation products go through the capillary opening of the patron as a vapour beam. This condenses at the thin layer plate as a start point and can then be chromatographed.

In fact, the thermofractography (TASOMAT) (16) offers the possibility of examining sublimable and unstable substances at a gradient temperature using a nitrogen stream. The sublimate condenses as a band on a thin layer plate.

About 10 mg of *Nigella sativa* L. powdered seeds was inserted in the TAS patron together with one molecular sieve 5 Å at a temperature 220° for 75 seconds. After development of thin layer plate with dichloromethane (double run) and visualization with anisaldehyde-sulphuric acid reagent, 7 spots were observed (R_f 0.96, 0.83, 0.74, 0.69, 0.59, 0.49 and 0.12, respectively). Thymoquinone with R_f 0.83 (major spot) gave violet colour after spraying with anisaldehyde-sulphuric acid reagent.

In the TASOMAT oven, about 10 mg of *Nigella sativa* L. powdered seed was heated at a linear temperature gradient from 50-330°C. After development with dichloromethane (double run) and spraying with anisaldehyde-sulphuric acid, the band corresponding to thymoquinone appeared at 200-330° and that corresponding to thymohydroquinone at 250-300°C.

GLC Technique :

GLC analysis was employed with 5% FFAP on Varoport 30 column (3 m X 32 mm), with the following conditions :

Detector	: flame ionization (FID)
Carrier gas	: helium
Flow rate	: 20 ml/min
Hydrogen flow rate	: 30 ml/min
Air flow rate	: 300 ml/min

Temp. programmed : from 100-200°
raised 6° / min.

Attenuation : 1x10⁻¹⁰

Chart speed : 1 cm/min

Isolation of thymoquinone by C.C. :

Volatile oil was fractionated on silica gel column (100g, 50x3 cm). The column was eluted with pet. ether, pet. ether-benzene (9:1), pet. ether-benzene (1:1), benzene and benzene-methanol (9:1) and 25 ml fractions were collected. Fractions obtained from pet. ether-benzene (9:1) gave one spot (substance A) with R_f 0.91 (benzene-ethyl acetate 85:15). This forms a crystalline derivative with 2,4 dinitrophenyl hydrazine(17).

Isolation of thymohydroquinone by chemical method :

About 20 ml of volatile oil was shaken with 50 ml sodium bicarbonate (5%). The aqueous layer was separated and the oily layer treated with 20 ml 5% sodium hydroxide. The aqueous layer was rendered acidic with dilute hydrochloric acid and extracted with ether. The ethereal extract was concentrated and upon cooling a crystalline substance was obtained (substance B) with (R_f 0.51) system : benzene-ethyl acetate (85:15). It gave green colour with ferric chloride solution and red colour with 5% alkali hydroxide .

Antimicrobial activities :

Both expressed and volatile oils of *Nigella sativa* L. seeds were tested for antimicrobial activity, adopting the disc agar diffusion method(18,19) using the available microorganisms. The bacteria were cultured on nutrient agar, while the fungi were cultured on Sabouraud-dextrose agar. Both types of organisms were inoculated on the surface of the agar plates. Paper discs of 6 mm diameter, were impregnated with 30µg/disc of the aqueous suspension of the expressed

Table (1) : Detected components by GLC in *Nigella sativa* L. volatile oil.

Peak No	Authentic	t _R linalool	Area %
1	α-pinene	0.24	13.60
2	β-pinene	0.27	5.20
3	sabinene	0.30	0.80
4	myrcene	0.32	0.20
5	unknown	0.34	0.20
6	limonine	0.37	3.60
7	1,8 - cincole	0.50	0.20
8	α-terpinene	0.57	0.90
9	p-cymene	0.60	37.50
10	unknown	0.62	0.09
11	artemisia ketone	0.70	0.40
12	sabinene hydrate	0.87	0.04
13	camphor	0.90	0.09
14	linalool	1.00	0.08
15	β-thujone	1.12	0.08
16	unknown	1.70	0.90
17	bornyl acetate	1.82	0.80
18	borneol	1.90	0.04
19	carvone	2.80	0.40
20	thymoquinone	3.00	30.90
21	thymol	3.40	0.04
22	carvacrol	3.64	3.00
23	thymohydroquinone	4.00	0.90

and volatile oils. The impregnated discs were applied on the surface of the inoculated plates. Then the plates were incubated at 35° (24 hrs.) for bacteria and 25° (48 hrs.) for fungi. The results are recorded in Table 2.

Investigation of the lipid constituents :

The lipids portion (ca 33%) extracted by petroleum ether from the seeds was saponified (20) and the unsaponifiable matter was fractionated on a neutral alumina column. The effluents were monitored on silica gel TLC using benzene-ethyl acetate (86:14) as a mobile phase. The column was developed by gradient elution technique. α-amyrin, β-sitosterol and oleanolic acid were isolated and identified through direct com-

paring their ir, mixed m.p and co-TLC with those of authentic substances.

The fatty acids recovered from the saponifiable fraction were methylated⁽²¹⁾ and the methyl esters were analysed by GLC. The chromatogram revealed that the saponifiable fraction is composed of:

- stearic : (ca 47%),
 - arachidic : (ca 33%),
 - palmitic : (ca 6.7%),
 - myristic : (ca 3.7%),
 - palmo-oleic : (ca 3.7%),
 - oleic : (ca 2.3%)
- and behenic : (ca 2.3%) acids.

Biochemical and Histopathological Study :

Ten mice from each group were sacrificed in the third day post-irradiation. Liver, intestine and kidney were excised. Livers were washed, dried, weighed and homogenized in ice cold distilled water to yield 10% homogenates, which were subjected to the following investigation :

Alkaline phosphatase; acid phosphatase according to Fujita (22); Acid ribonuclease and glucose-6-phosphatase according to the method of De Dove et al. (23); 5-nucleotidase and adenosine triphosphatase according to the method of Fisk and Subbarow (24) as modified by El-Aaser and El-Merzabani (25).

Histopathological Method :

The excised livers, kidneys and intestines were formalin fixed and paraffin embedded. The sections obtained were 4 microns thick and stained by haematoxylin and eosin for histopathological inspection.

RESULTS AND DISCUSSION

The volatile oil percentage of *Nigella sativa* varied according to the procedure used. Volatile oil prepared by gravimetric method by Karlsruher apparatus (14) was found to be 0.72% w/w, while that prepared by volumetric method was 0.4% v/w. However, the percentage of the volatile oil prepared by distillation of expressed oil attained 1.1% v/w. Comparing the above results revealed that the gravimetric method is more advantageous.

Concerning the constituents of the volatile oil, it was found that it contained a high carbonyl content and a low acids and esters. TLC and TAS investigations of the volatile oil revealed the presence of two different carbonyl compounds (R_f 0.15 and 0.91).

In the TASOMAT analysis the

band corresponding to thymohydroquinone appeared at 250-300°C and that of thymoquinone at 200-330°C, using column chromatography yellow plates (m.p. 45°; R_f 0.91) (substance A) were obtained. It formed crystalline derivative with 2,4-dinitrophenylhydrazine (m.p. 175°C) and with sodium bisulfite (m.p. 217°C). This substance was identified as thymoquinone (m.p, mixed m.p and IR) by comparison with authentic thymoquinone.

Fractionation of the oil with 5% NaOH yielded long white needle crystals of a phenolic compound, m.p. 137°C (substance B) which was identified as thymohydroquinone (m.p, mixed m.p and IR) by direct comparison.

The GLC study showed that the main constituents of the volatile oil are : p-cymene (31.5%), thymoquinone (30.9%), α -pinene (13.6%), β -pinene (5.2%), limonene (3.6%), carvacrol (3%) and thymohydroquinone (0.9%). These results differ qualitatively and quantitatively from previous results(13).

The results of the antimicrobial and antifungal activities demonstrated that the expressed oil (fixed and volatile oil)

Table (2) : Results of antimicrobial and antifungal screening of the expressed and volatile oils of *Nigella sativa* L. seeds

Microorganism	Expressed oil	Volatile oil
Staph. aureus	+++	+
Micrococcus spp.	++++	+
Streptococcus spp.	+++	+
Echerichia coli	++	-
Pseud. aeruginosa	+	-
Proteus vulgaris	+	-
Candida albicans	+++	-
Pencillium spp.	+++	-
Aspergillus spp.	+++	-

had a more pronounced antimicrobial activity than the distilled oil. The spectrum of antimicrobial activity covered the Gram + ve, Gram -ve bacteria, *Candida* and filamentous fungi. This may suggest that the antimicrobial substances seem to be the long chain fatty acids and the phenolic constituents which are abundant in both fixed and volatile fractions of the oil.

The study of lipid content in *Nigella sativa* L. seeds revealed the presence of: α -amyrin, β -sitosterol and oleanolic acid. Identification was confirmed by m.p., mixed m.p. and IR with the corresponding authentic substances.

GLC of the fatty acids esters revealed the presence of two major compounds: stearic acid (47.45%) and arachidic acid (32.01%). These essential fatty acids regulate the biosynthesis of prostaglandins required for various physiological functions in the body, through their effect on synthetase. On the other hand, they affect cyclooxygenase enzyme and suppress agents responsible for pain and inflammations in animal/or human organs.

Irradiation induced a significant decrease in the activity level of liver acid phosphatase and glucose-6-phosphatase. Meanwhile, acid ribonuclease, alkaline phosphatase, adenosine triphosphatase and 5-nucleotidase were significantly increased (Table 3).

In all animal groups administered *Nigella sativa* L. oil alone, no significant changes were observed in all enzymes activity compared with the untreated control group. The mean values of the enzyme activity level of animals treated with *Nigella sativa* L. oil and irradiated were non significantly decreased in glucose-6 phosphatase, acid phosphatase, acid ribonuclease and adenosine triphosphatase (94%, 91.8%, 91.6% and 97%, respectively). In case of alkaline phosphatase and 5-nucleotidase, although *Nigella sativa* L. oil had minimized the difference in the enzyme activity due to total body irradiation but this difference

remains still high (125.3% and 148.8%, respectively).

The radiated liver showed nuclear changes in the form of increased binuclear figures, mild atypia and cloudy swelling. Liver tissues also showed areas of focal macrocyst and focal lymphocytic cellular infiltration.

The radioprotective effect of *Nigella sativa* L. oil showed marked reduction of forementioned features, namely binucleation focal areas of necrosis and cloudy swelling; with the persistence of nuclear atypia and aberrant nuclei.

Liver tissues of mice treated with *Nigella sativa* L. oil alone showed similar normal morphological features of the control group except for the presence of focal areas of lymphocytic infiltration.

The effects of radiation on the kidney were in the form of areas of cloudy swelling, with congested glomeruli. Two out of five of kidney organs showed dense interstitial chronic in flowmeter cellular infiltrate.

On the other hand, the oil-treated irradiated kidneys showed no significant morphological changes. Also, the oil treated mice manifested interstitial inflammatory cellular infiltrate.

Irradiated intestines manifested shortening and broadening of intestinal villi associated with marked decrease in the number of goblet cells (mucus secreting cells). On the other hand, the radioprotective effect of *Nigella sativa* oil showed minimal morphological changes in the form of regaining the normal number of goblet cells, while, the administration of *Nigella sativa* L. oil alone proved similar morphologic features of the control group other than focal inflammatory cellular infiltrate.

The accumulation of lipids in organisms subjected to whole body irradiation, which has been proved by many authors (10), was explained as a trial done by the organisms to recover from radiation injury. This phenomenon encour-

aged many investigators to examine the radioprotective effect of some oil e.g olive oil (26,27).

In the present study *Nigella sativa* L. oil has been tested as radioprotective agent. The changes in the enzymatic activity levels of different enzymes localized in the different subcellular structures had been measured. This attempt aimed evaluating the extent of injury and protection of these subcellular structure after exposure to ionizing radiation with and without *Nigella sativa* oil. Moreover, the enzymatic changes rather than the cellular morphology proved the radioprotection in the liver treated with *Nigella sativa* oil. The changes in the activity levels of cell membrane enzymes namely : alkaline phosphatase and 5-nucleotidase due to the effect of radiation on the hepatic tissue are in agreement with that reported by other investigators (27,28).

Obviously, irradiation induced significant decrease in acid phosphatase and significant increase in the acid ribonuclease which are lysosomal enzymes(11,29).

The histopathologic changes after

irradiation showed variable results among the different organs studied. Thus, the liver showed marked irradiation changes, whereas the intestine and kidney changes were less remarkable compared to that observed in the liver. This difference in irradiation effect may be contributed to the variability of cell division and turn-over among the tissues studied.

The present report also shows that the radioprotective effect of *Nigella sativa* oil on liver was on the morphologic level, in the form of marked reduction in nuclear appression and necrosis. On the other hand, the intestine showed minimal cytomorphologic difference in the oil-treated and untreated irradiated mice.

Although, the kidney is a multi-functional organ, yet there were no significant morphologic changes detected in oil treated irradiated and untreated mice. So, the reflection of kidney functions on morphologic grounds using light microscopy may not be evident. However, it may be detected on biological ground, cell proliferation, activation and DNA ploidy.

Table (3) : Enzyme activity in the liver tissue homogenate in normal, irradiated and irradiation-protected mice.

Group	Glucose 6-phosphatase	Acid Ribonuclease	ATP-ase	5-Nucleotidase	Alkaline phosphatase	Acid phosphatase
Control	7.25±0.29	7.33±0.61	8.11±0.12	4.34±0.25	2.11±0.22	3.93±0.12
Irradiated	5.10±0.37**	11.19±0.43***	10.98±0.62**	7.22±0.43***	4.31±0.34***	2.64±0.41***
N. oil	7.30±0.61	7.41±0.63	7.63±0.51	4.91±0.31	1.97±0.61	3.81±0.44
N. oil + irradiation	6.85±0.55	6.72±0.51	7.87±0.46	5.44±0.63	3.14±0.00*	3.61±0.24

* P < 0.05 significant

*** P < 0.01 highly significant

** P < 0.01 significant

Results are expressed as X ± S.E.

Both the present investigation as well as the previous studies (26,27,30), indicated high sensitivity of enzymatic assays in monitoring cell damage. Administration of *Nigella sativa* oil as source of unsaturated fatty acids might have a repair effect, may produce peroxides which start a chain of autooxidation and free radical formation leading to the transfer of electron to disulphide groups of any other antioxidant (26). Results also showed that *Nigella sativa* oil acted as protective agent for whole body irradiated mice.

However, further investigations are required before extrapolating these findings to clinical use. The relative safety of these herbal drugs strongly encourages further use for human, but this necessitates the determination of the most effective dosage.

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دراسة الزيت الطيار والدهون الموجودة في بذور نبات حبة البركة (لينيه) والمسح البيولوجي لها

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تم في هذا البحث دراسة الزيت الطيار لبذور نبات حبة البركة (لينيه) التي تنمو في مصر وقد تم تعيين نسبة الزيت باستخدام الطرق المختلفة وكذلك تحليل الزيت الطيار باستعمال كروماتوجرافيا الطبقة الرقيقة ، والتحليل الحراري للزيت وكذلك كروماتوجرافيا الغاز وتم تعيين الخواص الطبيعية وثوابت الزيت .
وقد وجد أن عينة الزيت الطيار للبذور تحتوي على ٢٣ مركبا كان اهمها السيمييين (٣٧.٥٪) ، الفابييين (١٣.٦٪) ، الثيموكيبتون (٣.٩٪) وقد عمل مسح ميكروبيولوجي للزيت الطيار وكذلك المحضر من عصر البذور ووجد أن الزيت المعصور له تأثير على البكتريا والفطريات يفوق الزيت الطيار .
وتم تحليل المواد الغير متصينة الموجودة في بذور حبة البركة (لينيه) وتم فصل والتعرف على كل من الفا اميرين ، بيتا سيتوستيرول وحمض الأوليانوليك كما تم دراسة الاحماض الدهنية المحضرة من بذور حبة البركة وقد تم التعرف على ٨ مركبات اهمها حامض الاستياريك (٤٧٪) ، حامض الاراشيدك (٣٣٪) ، حامض البلميتيك (٧٪) وحمض الميرستيك (٤٪) .
وتم دراسة التأثير الواقى لزيت حبة البركة على انسجة الثدييات التي تم تعرضها للاشعاع وقد وجد أن الزيت يمكن أن يعيد التوازن للانزيمات التي تأثرت بالاشعاع في انسجة الكبد وكذلك وجد أن للزيت تأثير واضح على شكل خلايا الكبد والامعاء ، ولكن لم يلاحظ تأثير واضح على خلايا الكلى .