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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF BLACK SEED OIL (*NIGELLA SATIVA* L.)

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Keywords:

Nigella sativa, Ranunculaceae, 9-eicosyne, fatty acid, antioxidant activity

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ABSTRACT: *Nigella sativa* L. is an annual herb and cultivated largely in the East Mediterranean region. Seeds used in traditional folk medicine for the treatment of various purposes in the systems of Unani, Ayurveda, Chinese and Arabic. *Nigella sativa* seed oil was isolated using soxhlet hexane extraction process. GC-MS analysis identified a total of 32 compounds among which 9-eicosyne (63.04%), linoleic acid (13.48%), palmitic acid (9.68%) were the major constituents. Saturated aliphatic fatty acid accounted 63.04% of the seed oil extract. Fatty acid and monoterpene hydrocarbon constituted 23.26% and 4.91% respectively. Also the seed oil included compounds of alkanes and sesquiterpene hydrocarbons that constituted 2.84%, and 0.30% respectively. The seed oil was estimated for its chemical compounds and antioxidant activity using *in vitro* assays such as DPPH, ABTS, nitric oxide, hydrogen peroxide and total antioxidant scavenging capacity. Higher antioxidant scavenging activity of TAC and ABTS was found in seed oil. The seed oil contains higher percentage of fatty acids and exhibit antioxidant activity which are useful for preparation of pharmaceutical products

INTRODUCTION: *Nigella sativa* L. is an annual herb belonging to the Ranunculaceae family and cultivated in various parts of the globe, in particular in the East Mediterranean region [1]. Seeds of *Nigella sativa* have been used in traditional folk medicine for the treatment of various purposes in ancient medical systems of Unani, Ayurveda, Chinese and Arabic for quite long time [2]. The extracts of seeds have been reported to possess anti-inflammatory and antioxidant activities, and also suppress coughs, disintegrate renal calculi, retard carcinogenic process, treat abdominal pain, diarrhea, flatulence and polio [3, 4].

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Activity of ingredients was reported in *Nigella sativa* seeds against various types of cancers including cervical [5], blood [6], hepatic [7], colon [8], skin [9], fibrosarcoma[10], renal [11], prostate [12] and breast [3].

Nigella sativa seed contains fixed oil that ranges between 28 to 36% and chiefly composed of unsaturated fatty acids that are arachidonic, eicosadienoic, linoleic and linolenic and saturated fatty acids that includes palmitic, stearic and myristic [13]. The seed oil contains compounds such as cholesterol, campesterol, stigmasterol, β-sitosterol, α-spinasterol, (+)-citronellol, (+)-limonene, p-cymene, citronellyl acetate, carvone, nigellone, arachidic, linolenic, linoleic, myristic, oleic, palmitic, palmitoleic and stearic acids [14]. Seed oil contains fixed oils like linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%) and volatile oils like trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%) [15].

A literature search revealed that a complete chemical profiling of *Nigella sativa* seed oil has not yet been reported. The present investigation was therefore undertaken to obtain analyse the chemical composition of *Nigella sativa* seed oil and its antioxidant activities.

MATERIALS AND METHODS:

Collection of seed:

Seeds of *Nigella sativa* were purchased from the herbal plant and powder shop in Triplicane, Chennai, Tamil Nadu, India. The seed material was sieved and false and small seeds and inert material removed.

Extraction of seed oil:

The seeds were coarsely ground using a table top mixture and seed oil extracted using hexane in a soxhlet apparatus for 2 hours and stored in an amber glass screw cap bottle at room temperature until use.

Gas Chromatography-Mass Spectrometry analysis:

GC-MS analysis were conducted using Agilent MSD (5975B-inert XL MSD) apparatus equipped with reference libraries (NIST); column DB-5MS (J&W Scientific) cross-linked fused-silica capillary column (30 m × 0.25 mm × 0.25 µm thickness), coated with 5% phenyl-polymethylsiloxane; column temperature, 80°C for 0 min, rising to 150°C at 10°C/min, then 250°C at 5°C/min, then rising to 270°C at 20°C held for 6 min. injector temperature 270°C, injection mode, split; split ratio 1:20; volume injected, 2 µl of the seed oil. Helium was used as a carrier; interface temperature 270°C; acquisition mass range, m/z 55-550. The compounds of the oil were identified by comparing their retention indices (RI), with NIST (National Institute of Standards and Technology) library.

Antioxidant activities:

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) scavenging assay:

The ABTS radical cationdecolorization method is based on the reduction of ABTS•+ radicals by antioxidants of the essential oil tested. The tubes containing ABTS and APS were incubated at room temperature for a period of 16 hours. 20 µl of various concentrations of 10 mM PBS pH 7.4 test solutions was added to 230 µl of ABTS radical solution (0.238 mM). The absorbance values were recorded immediately at 734 nm using shimadzu UV 1800 spectrophotometer [16].

ABTS scavenging activity (%)= $[A_{control} - A_{sample}/ A_{control}] \times 100$

DPPH (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging assay:

Free radical scavenging activity of the seed oil was measured in terms of radical scavenging ability using the stable free radical DPPH [16]. Different concentrations (10µl, 20µl, 30 µl, 40µl & 50µl) of sample were taken and 50µl of 0.659 mM DPPH dissolved in methanol solution was added to make up to one using double distilled water. The tubes were incubated at 25ºC for 20 minutes.The absorbance value was recorded at 510 nm using shimadzu UV 1800 spectrophotometer. The same procedure was followed for control without the sample.

DPPH Scavenging ability (%) = $[A_{control} - A_{sample}/ A_{control}] \times 100$

Hydrogen peroxide radical scavenging assay:

The ability of the oil to scavenge hydrogen peroxide was determined according to the method described by Rajamanikandan et al [17]. 0.6ml of 40mM of hydrogen peroxide was prepared using 50mM phosphate buffer (pH 7.4).Different concentrations (10µl, 20µl, 30 µl, 40µl & 50µl) of sample were added to hydrogen peroxide solution.The tubes were incubated for 10 minutes. The absorbance values were recorded at 230 nm using shimadzu UV 1800 spectrophotometer.

Hydrogen peroxide activity (%) = $[A_{control} - A_{sample}/ A_{control}] \times 100$

Nitric oxide scavenging assay:

Nitric oxide scavenging assay was determined using method by Jain & Agrawal [16]. Different concentrations (10µl, 20µl, 30 µl, 40µl & 50µl) of sample were taken and added 50µl of 10mM sodium nitroprusside dissolved in 0.5M phosphate buffer (pH 7.4). The tubes were incubated under fluorescent light at room temperature for 15 minutes. After the incubation period, 125 µl of

Griess reagent was added [Griess reagent: 0.1% of N-1-Naphthyl-ethylene diamine dissolved in water, 1% Sulphanilic acid dissolved in 5% Orthophosphoric acid]. The tubes were incubated again at room temperature for 10 minutes. The absorbance values were recorded at 546 nm using shimadzu UV 1800 spectrophotometer.

Total antioxidant capacity assay:

Total antioxidant capacity assay was determined as described by Rajamanikandan et al [17]. Different concentrations (10µl, 20µl, 30 µl, 40µl & 50µl) of extracts were taken and 1ml of reagent solution was added.[Reagent solution: 0.6M sulphuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate].

The tubes were capped and incubated in thermal block at 95ºC for 90minutes. After the time interval the tubes were cooled down at room temperature. The absorbance was recorded at 695 nm using shimadzu UV 1800 spectrophotometer.

Statistical analysis:

Results were documented as mean ± standard deviation (n = 3) and focused on one-way analysis of variance (ANOVA). The significance of the difference between means was determined by Duncan's multiple range test ($P<0.05$) using SPSS 17.0 statistical software (SPSS South-Asia Pvt Ltd, Bangalore).

RESULTS AND DISCUSSION:

Chemical constituents of *Nigella sativa*:

GC-MS chemical profile of the hexane extract of seed oil contains a total of 32 compounds and 9-eicosyne (63.04%) was a major chemical constituent present in *Nigella sativa* seed oil. The other chemical constituents present were linoleic acid (13.48%) followed by palmitic acid (9.68%), p-cymene (2.54%) and thymoquinone (1.86%) and cs-7-dodecen-1-yl acetate (1.11%) **(Table1)**. The chromatogram of GC-MS chemical profile of seed oil is presented in **Fig. 1**.

TABLE 1: GC-MS CHEMICAL CONSTITUENTS OF *NIGELLA SATIVA* SEED OIL

S.no.	Retention Time	Chemical Name	Area %
1	2.016	2-Methylpentane	0.38
2	2.087	3-Methylpentane	0.39
3	2.414	Methylcyclopentane	0.88
4	2.761	Cyclohexane	0.52
5	9.277	4-methyl-1-(1-methylethyl),didehydro-biocyclo[3.1.0]hexane	0.67
6	9.440	(+)-α-Pinene	0.62
7	10.349	Sabinene	0.38
8	10.441	(-)-β-Pinene	0.37
9	11.442	P-Cymene	2.54
10	11.503	(-)-Limonene	0.35
11	12.055	γ-Terpinene	0.39
12	12.719	β-Terpinene	0.26
13	13.127	4,6-Diamino-5-formamidopyrimidine	0.33
14	13.883	Phellandral	0.13
15	14.223	Terpinen-4-ol	0.24
16	15.292	Thymoquinone	1.86
17	16.171	P-Thymol	0.07
18	16.742	(+)-α-Longipinene	0.06
19	17.702	Longifolene	0.24
20	20.419	Paeonol	0.11
21	23.248	(R)-(+)-β-Citronellol	0.05
22	23.850	Myristic acid	0.10
23	27.292	3-Todomethyl-3,6,6-trimethyl-cyclohexene	0.11
24	27.445	Cyclododecene	0.19
25	27.905	Palmitic acid	9.68
26	29.774	Methyl linoleate	0.14
27	29.917	3,5-Dimethylcyclohexanol	0.91
28	31.459	9-Eicosyne	63.04
29	31.643	Linoleic acid	13.48
30	34.226	Cs-7-Dodecen-1-yl acetate	1.11
31	35.493	Octadeca-9,17-dienal	0.11
32	36.146	Linoleic acid ethyl ester	0.29

Saturated aliphatic fatty acid	63.04%	Alkanes hydrocarbon	2.84%
Fatty acid	23.26%	Sesquiterpene hydrocarbon	0.30%
Monoterpene hydrocarbon	4.91%	Others	5.65%

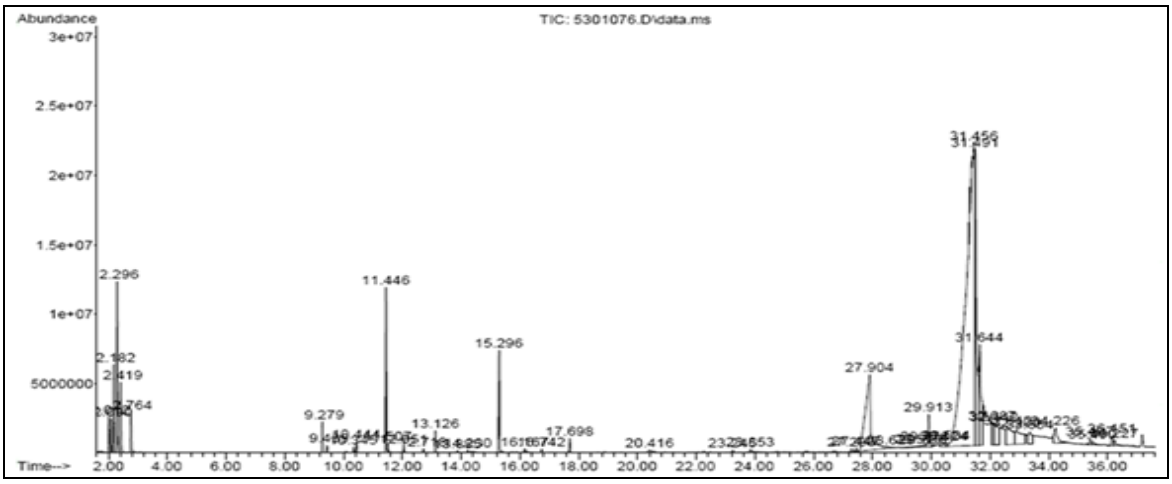

FIG. 1: GC-MS CHROMATOGRAM OF SEED OIL OF *NIGELLA SATIVA*

The present report of seed oil contains higher percentage of 9-eicocyne (63.04%). Similarly, 9-eicosyne compound has been reported from other plant species 23.64% in *Blepharismaderaspatensis* [18], 19.61% in *Borassus flabellifer* [19], 11.91-23.32% in *Portulacaoleracea* [20], 5.30% in *Stevia rebaudiana* [21], 2.74% in *Syzygium calophyllifolium* [22], 2.24% in *Melia azedarach* [23], 1.74% in *Cassia auriculata* [24], 1.47% in *Andrographis paniculata* [25], 0.756% in *Buxus microphylla* [26], 0.36% in *Atractylodes macrocephala* [27]. Thymoquinone is an important bioactive constituent of the volatile oil of *Nigella sativa* and reported to exert several pharmacological activities such as antioxidant, antihistaminic, chemotherapeutic and anti inflammatory activities [28, 29, 30, 31, 32].

Major group of components were present in the seed oil and included aliphatic fatty acid (63.04%), fatty acid (23.26%), monoterpene hydrocarbon (4.91%), alkanes hydrocarbon (2.84%) and sesquiterpene hydrocarbon (0.30%) (**Table1**). *Nigella sativa* seed oil contains anethole, p-cymene, limonene, carvone and thymoquinone[15]. Seed oil consists of four saturated fatty acids (17.0%) and four unsaturated fatty acids (82.5%). Linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%) are its major components [15]. The present investigation of *Nigella sativa* seed oil posses higher percentage of fatty acids (86.30) and few chemical compounds are also similarly reported fatty acids [33] and other chemical constituents [34] but the percentages of chemical composition differs.

Antioxidant activities:

The present analysis indicates that higher composition of fatty acids (86.30%) was present in the hexane seed oil extract. Fatty acids are known as important nutrients in both human and animal diets, and also possess various health benefits [35, 36], and are used in the pharmaceutical industry [37]. Saturated and unsaturated fatty acids from various seed oil sources showed good antioxidant activities [38]. Seed oil of *Nigella sativa* was analyzed for antioxidant assays. Higher ABTS scavenging activity was found in 10 and 20 µg/ml of lower concentration of seed oil **(Fig. 2A)**.

The activity of seed oil decreases proportionally with the concentration of the sample and is comparable with that of the standard. Seed oil of *Nigella sativa* showed scavenging activities of DPPH, H_2O_2 and NO that increased scavenging activity in lower concentration and decreases in higher concentration **(Fig. 2B, 2C and 2D)**. Seed oil of *Nigella sativa*is capable of scavenging higher percentage of TAC molecule compared to ascorbic acid **(Fig. 2E)** and also higher scavenging activity compared to ABTS.

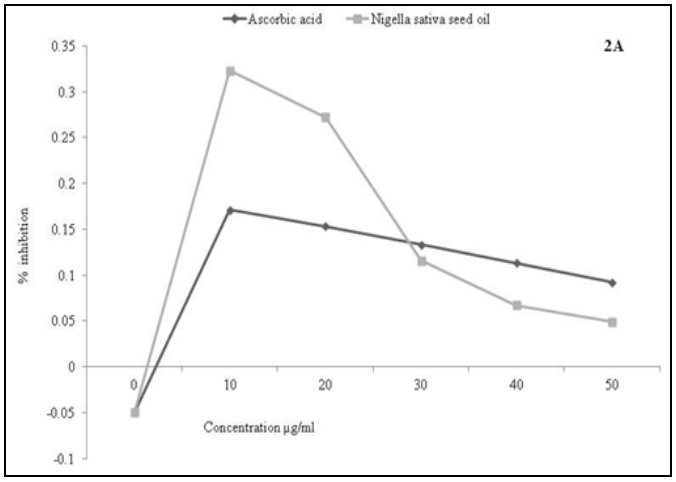

FIG. 2A: ABTS SCAVENGING OF HEXANE EXTRACT OF *NIGELLA SATIVA* SEED OILCOMPARED TO THAT OF ASCORBIC ACID. EACH VALUE IS EXPRESSED AS MEAN ± STANDARD DEVIATION (n=3)

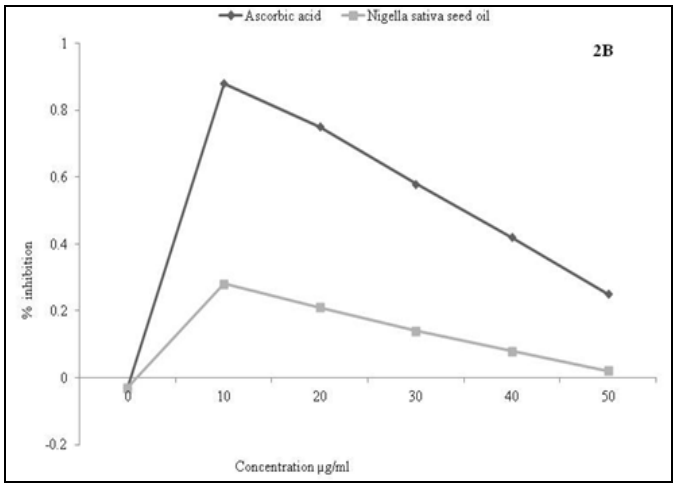

FIG. 2B: DPPH SCAVENGING OF HEXANE EXTRACT OF *NIGELLA SATIVA* SEED OILCOMPARED TO THAT OF ASCORBIC ACID. EACH VALUE IS EXPRESSED AS MEAN ± STANDARD DEVIATION (n=3)

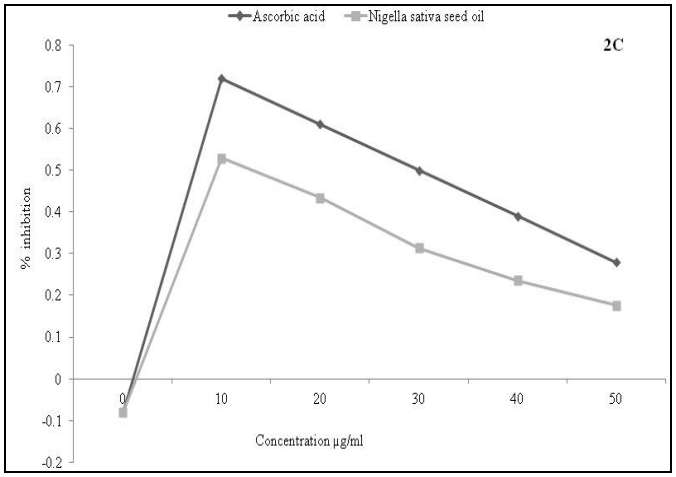

FIG. 2C: HYDROGEN PEROXIDE RADICAL SCAVENGING OF HEXANE EXTRACT OF *NIGELLA SATIVA* SEED OIL COMPARED TO THAT OF ASCORBIC ACID. EACH VALUE IS EXPRESSED AS MEAN ± STANDARD DEVIATION (n=3)

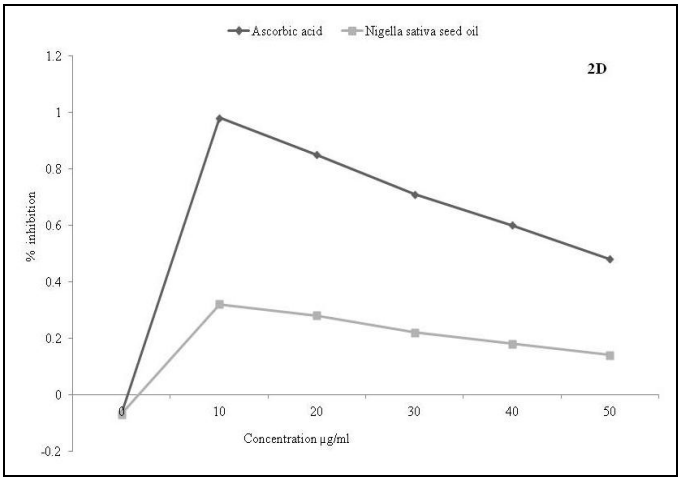

FIG. 2D: NITRIC OXIDE SCAVENGING OF HEXANE EXTRACT OF *NIGELLA SATIVA* SEED OIL COMPARED TO THAT OF ASCORBIC ACID. EACH VALUE IS EXPRESSED AS MEAN ± STANDARD DEVIATION (n=3)

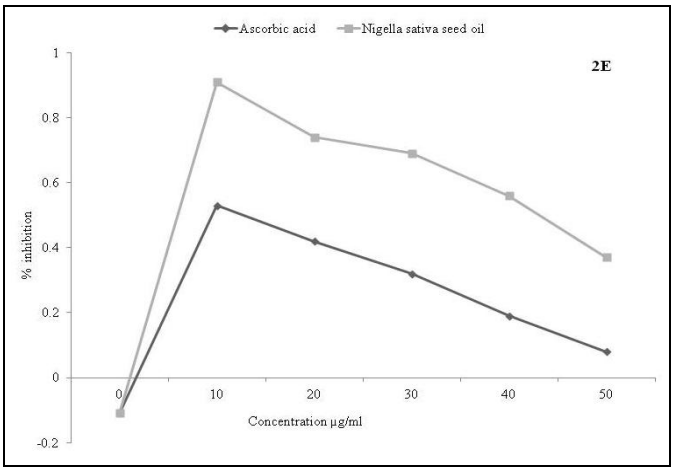

FIG. 2E: TOTAL ANTIOXIDANT SCAVENGING OF HEXANE EXTRACT OF *NIGELLA SATIVA* SEED OIL COMPARED TO THAT OF ASCORBIC ACID. EACH VALUE IS EXPRESSED AS MEAN ± STANDARD DEVIATION (n=3)

All assays uniformly showed that higher scavenging activity was found in lower concentrations of the seed oil (10 and 20 µg/ml) and increasing concentrations (30, 40 and 50 µg/ml) showed decreased scavenging activities. The hexane extract of *Nigella sativa* seed oil showed higher radical scavenging activity in lower concentration against ABTS and TAC free radical. The present analysis of seed oil contains 1.86% of thymoquinone. The thymoquinone (20.32%) exhibited strong antioxidant activity (14.0±0.7 µg/ml) in essential oil and has been found to be the most active compound to decrease oxidation and NO excretion [39], anti-eicosanoid and antioxidant activity [33]. Also higher composition of fatty acids plays higher scavenging biological activities.

Consumption of fatty acids as a dietary supplement or as a food ingredient has the potential to provide health benefits [40].

CONCLUSION: From the present study *Nigella sativa* seed oil is found to contain higher percentage of fatty acid and higher scavenging activities in lower concentrations. There is a need for further exploration for their medicinal properties and utilization.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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