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SPECTRAL ANALYSIS OF SEED EXTRACT OF KALONJI (*NIGELLA SATIVA*) USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND MEDICINAL POTENTIAL : AN OVERVIEW

Manisha* and Dr. Mahesh C. Sharma

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Abstract

Nigella sativa linn commonly known as kalonji or black seed, member of Ranunculaceae family, widely used in various traditional medicinal systems like Ayurveda and Siddha. Its Seed and seed oil has been considered as diuretic, carminative, vermifuge and lactogogue. Extensive pharmacological studies reveals the antimicrobial, anti-inflammatory, antidiabetic, immune potentiating, antitumor, antimicrobial, CNS depressant and antihistaminic activities of seeds extracts. Maximum therapeutic properties of black cumin are due to the presence of essential oil major component thymoquinone. The present study evaluate the phytoconstituents present in the pet ether, chloroform and acetone seed extracts. A total of 28, 13 and 17 components are identified from the gas chromatography and mass spectroscopy (GC-MS) analysis of pet ether, chloroform and acetone extracts of seeds respectively. The structure and formula of phytoconstituents present in different solvents predicted by the GC-MS reports.

Keywords: Nigella sativa, Ranunculaceae, Siddha, Phytoconstituents, GC-MS..

Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan INDIA 302004

Email ID: manialaria1989@gmail.com

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Introduction:-

WHO recommended that most of the world's population depends on the herbal medicine for their health care. *Nigella sativa* is the most common medicinal plant that used worldwide. It belongs to the Ranunculaceae family that consists of about 50-60 genera (1) and more than 2,000 known species (2). The genus *Nigella* comprises 20 species (3). *Nigella sativa Linn* is an annual herb native to S.W. Asia and cultivated in Europe and N. Africa (4). In India it is cultivated in Punjab, Himachal Pradesh, Bihar, Assam and Maharashtra (5).

It grows up to 20–30 cm height, leaves are linear, lanceolate and flowers colors are generally white, blue, yellow, purple and pink. The fruits are large and have numerous seeds. The black coloured seed has 0.2 cm length and 0.1 cm thickness. Seeds are angular, funnel, oblong and flattened (6).

The seed are medicinal food known as black cumin or black seed (English) or kalongi (hindi) (3), Al-Haba-El-Sauda or Habat-Al-Baraka (Arabic). Seeds has been used in folk medicine to treat bronchial asthma, diarrhea, rheumatism, hypertension, and gastrointestinal problems. Biological study of seed extract includes the antioxidant, antifungal, antiulcer, immunomodulatory, antihelminthic, anticarcinogenic and antiparasitic activity (7).

The black cumin possesses nutritional components like carbohydrates, protein, fat, fibre, vitamin A, protein, essential ammino acids, mineral elements like Iron, Potassium & Calcium(8-9) that protective against food poisoning(10).

Alkaloid, saponins, isobenzofuranone, flavonol triglycosides, unsaturated fatty acids have been isolated from black seed. Flavonoids and triterpenoid show antidiabetic activity (11). Thymoquinone, the major active compound shows the hepatoprotective effect against liver damage induced by CCl4(12-13). Seed oils are widely used as food flavours and preservatives. It increase the immunity and milk secretion in nursing mothers (14) and also considered as stimulant, diaphore4ie and emmenagogue(15).

Seeds contain alpha-hederin (pentacyclic triterpene saponin) have anticancer properties (13).

Bioactive compounds of seed α-hederin, nigellidine, thymoquinone are very effective in control of the COVID-19(16).

Major phenolic compound isolated from seed extract of kalonji are thymoquinone (TQ), dithymoquinone, thymohydroquinone and thymol. Thymoquinone has been shown the anti-inflammator, antioxidant, immunomodulatory and anti-cancer activities(17), anticonvulsive, anti arthritic(18), asthma alleviating and antiviral properties(19).

Estrogenic activity of Black seed was conformed by the study of ethanolic extract in male rats(10). Methanolic and aquous extract exhibit the antimicrobial effect (6).

Nigella sativa crude extract and n-hexane extract of seed confirm the protective effect in diabetes.

It is well known medicinal plant that used in traditional medicinal system like Ayurveda , Siddha, unani and Tibb(13). According to Ayurveda, black seed pacifies the Vata dosha, increases the Pitta dosha and reduces the Kapha dosha(5).

In bread, sauces, salads, marinades and yogurt the *N. sativa* seeds used as spices. marinades, sauces, and salads(20). The bioactive compounds reported in black seed are 4-terpineol, thymol, p-cymene, sesquiterpene longifolene, thymoquinone, α-pinene, carvacrol, dithymoquinone, thymohydroquinone, t-anethol etc.(21).

MATERIAL AND METHODS

Collection of Sample

N. sativa seeds were collected from Jaipur, Rajasthan (India) and were washed, cleaned and dried.

Preparation of extracts

A grinder was used to reduce the dried seeds of *N. sativa* to a fine powder. Then, the powder (100 g) was put in a soxhlet apparatus and successively extracted for 12 hours on a water bath with 500 ml petroleum ether, chloroform, and acetone. To eliminate impurities, the aforementioned extracted were filtered through Whatman No. 41 paper (Merck, Mumbai, India). A rotating evaporator used a vacuum to remove extra solvent. The GC-MS analysis of the concentrated extracts was then performed.

Instruments and Chromatographic Conditions

For routine compound analysis, gas chromatography with mass spectroscopy are a preferable technology. In this investigation, 1.5 µl of *N. sativa* petroleum ether, chloroform, and acetone extracts were employed independently for the GC-MS analysis in order to identify diverse phytochemical components. The Manipal University CAF Center in Jaipur used the GC-MS technology. The following circumstances were used during analysis utilising a gas chromatography unit Shimadzu GCMS-QP2020 instrument: equipped with the Rxi-5 capillary column (30m in length x 0.25µm in thickness x 0.25mm in diameter); He gas was used as carrier gas and an injection volume of 1.5µL was used (split ratio of 1.0) with column flow rate 1.18 mL/min; total flow rate 7.4mL/min; Injection Temp. 250°C; Ion source Temp. 250°C; Interface Temp. 250°C; Pressure at column inlet 66.8kPa. Initial column oven Temp. was 50ºC, held for 02min; finally programmed to 250ºC at a rate of 8ºC/min, then held for 02min, total program time 31.92min. The method of electron-impact ionisation was applied. All information was gathered by gathering full scan mass spectra between 40 and 550 m/z with a scan speed of 2000. Components are identified Different retention durations used by the mass spectrometer to detect the components allowed for the identification of various components. The software that was coupled to it created a plot of intensity against retention time known as a chromatogram. By comparing the data with the software library NIST17 and standard mass spectra, the chemicals in the graph are identified. The chemical structures of identified compounds based on retention time and peak area.

RESULT AND DISCUSSION

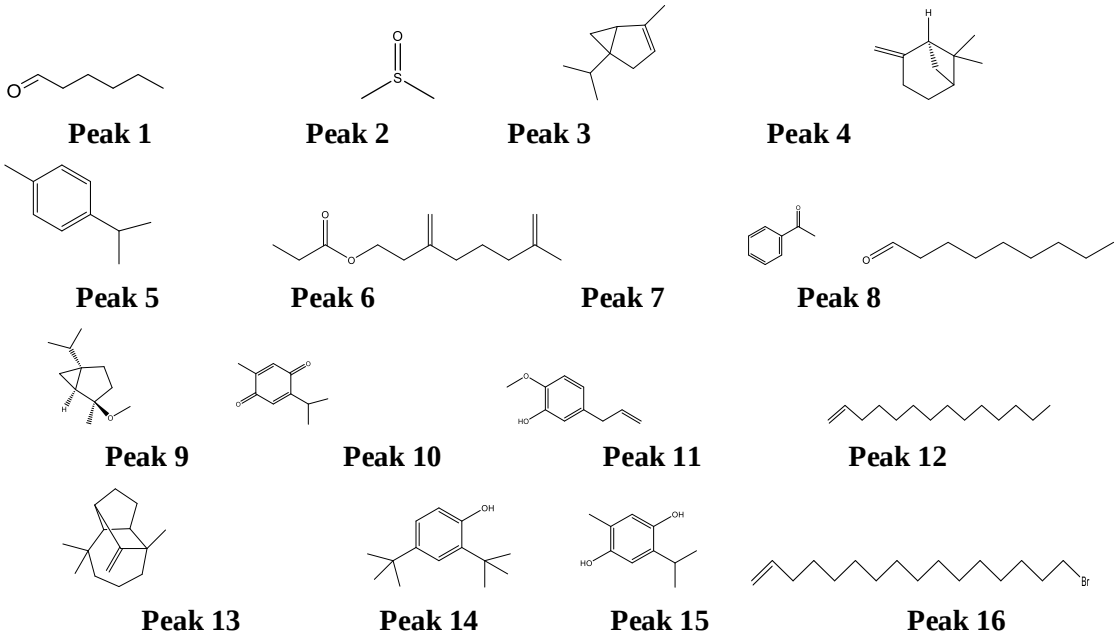
Chromatograms of the petroleum ether, chloroform, and acetone extracts identified 28, 23, and 17 chemicals, respectively. n-Pentadecanol (31.56%) had the highest content in the pet-ether extract's GC/MS profiles, followed by 9,12-Octadecadienoyl chloride (9.60%), 7-Hexadecenal (7.13%), and Heneicosanoic acid, methyl ester (6.66%). Phytoconstituents detected in the chloroform seed extract of the plant using gas chromatography-mass spectrometry are Isopropyl linoleate (39.15%), Linoleic acid ethyl ester (26.31%) and 9-octadecenoic acid, 2,2,2-trifluoroethyl ester (12.62%). Twentyone phytochemicals identified in the acetone extract. Major compounds are Glycidyl palmitate (26.06%), cis-9-Octadecenoic acid, propyl ester (22.79%), 1,8,11-Heptadecatriene, (Z,Z)- (12.20%).

Table 1: Phytochemicals discovered by GC-MS in the petroleum-ether extract of the *Nigella sativa* seed sample:

Peak#	R.Time	Area%	Mol. Weight	Mol. Formula	Compound Name
	4.089	0.71	100	$C_6H_{12}O$	Hexanal
2	5.227	1.24	78	C_2H_6OS	Dimethyl Sulfoxide
3	6.886	1.91	136	$C_{10}H_{16}$	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-
4	8.235	0.49	136	$C_{10}H_{16}$	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)
5	9.489	5.56	134	$C_{10}H_{14}$	p-Cymene
6	9.618	0.85	210	$C_{13}H_{22}O_2$	7-Methyl-3-methylene-7-octen-1-ol, propanoate (ester)
7	10.629	5.73	120	C_8H_8O	Acetophenone
8	11.601	1.57	142	$C_9H_{18}O$	Nonanal

9	12.020	0.90	168	$C_{11}H_{20}O$	(1R,4R,5S)-1-Isopropyl-4-methoxy-4-methylbicyclo [3.1.0] hexane
10	15.354	2.95	164	$C_{10}H_{12}O_2$	Thymoquinone
11	17.628	0.84	164	$C_{10}H_{12}O_2$	3-Allyl-6-methoxyphenol
12	18.305	0.85	196	$C_{14}H_{28}$	1-Tetradecene
13	18.841	0.73	204	$C_{15}H_{24}$	Longifolene
14	20.527	1.90	206	$C_{14}H_{22}O$	2,4-Di-tert-butylphenol
15	21.470	1.41	166	$C_{10}H_{14}O_2$	p-Cymene-2,5-diol
16	21.842	1.93	302	$C_{14}H_{31}Br$	1-Hexadecene, 16-bromo-
17	24.795	0.91	592	$C_{41}H_{84}O$	1-Hentetracontanol
18	24.890	1.68	310	$C_{16}H_2F_3O_2$	Tetradecyl trifluoroacetate
19	26.165	3.38	262	$C_{19}H_{34}$	E,Z-1,3,12-Nonadecatriene
20	26.698	1.21	270	$C_{14}H_{34}O_2$	Hexadecanoic acid, methyl ester
21	27.159	2.51	278	$C_{16}H_{22}O_4$	Dibutyl phthalate
22	27.601	2.25	284	$C_{19}H_{40}O$	n-Nonadecanol-1
23	27.681	0.95	352	$C_{25}H_{52}$	2-Methyltetracosane
24	27.993	9.60	298	$C_{18}H_{31}ClO$	9,12-Octadecadienoyl chloride, (Z,Z)-
25	28.860	2.61	308	$C_{20}H_{36}O_2$	11,14-Eicosadienoic acid
26	28.946	7.13	238	$C_{16}H_{30}O$	7-Hexadecenal, (Z)-
27	29.240	6.66	340	$C_{22}H_{44}O_2$	Heneicosanoic acid, methyl ester
28	30.069	31.56	228	$C_{15}H_{32}O$	n-Pentadecanol

Structure of phytochemicals determined by GC-MS in the petroleum-ether extract of an *Nigella sativa* seed sample:



Peak 1 **Peak 2** **Peak 3** **Peak 4**

Peak 5 **Peak 6** **Peak 7** **Peak 8**

Peak 9 **Peak 10** **Peak 11** **Peak 12**

Peak 13 **Peak 14** **Peak 15** **Peak 16**

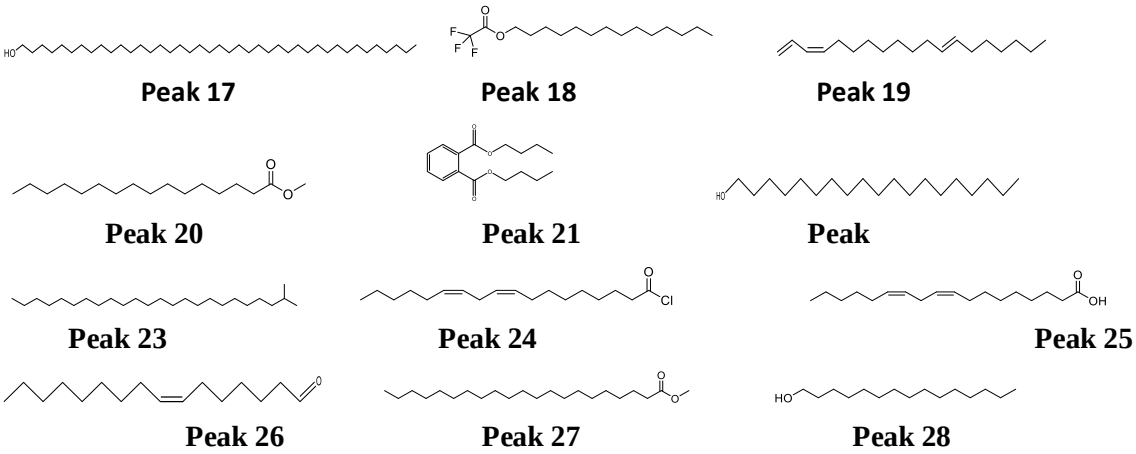


Table 2: Major Phytochemicals identified in petroleum-ether extract of *Nigella sativa* seeds:

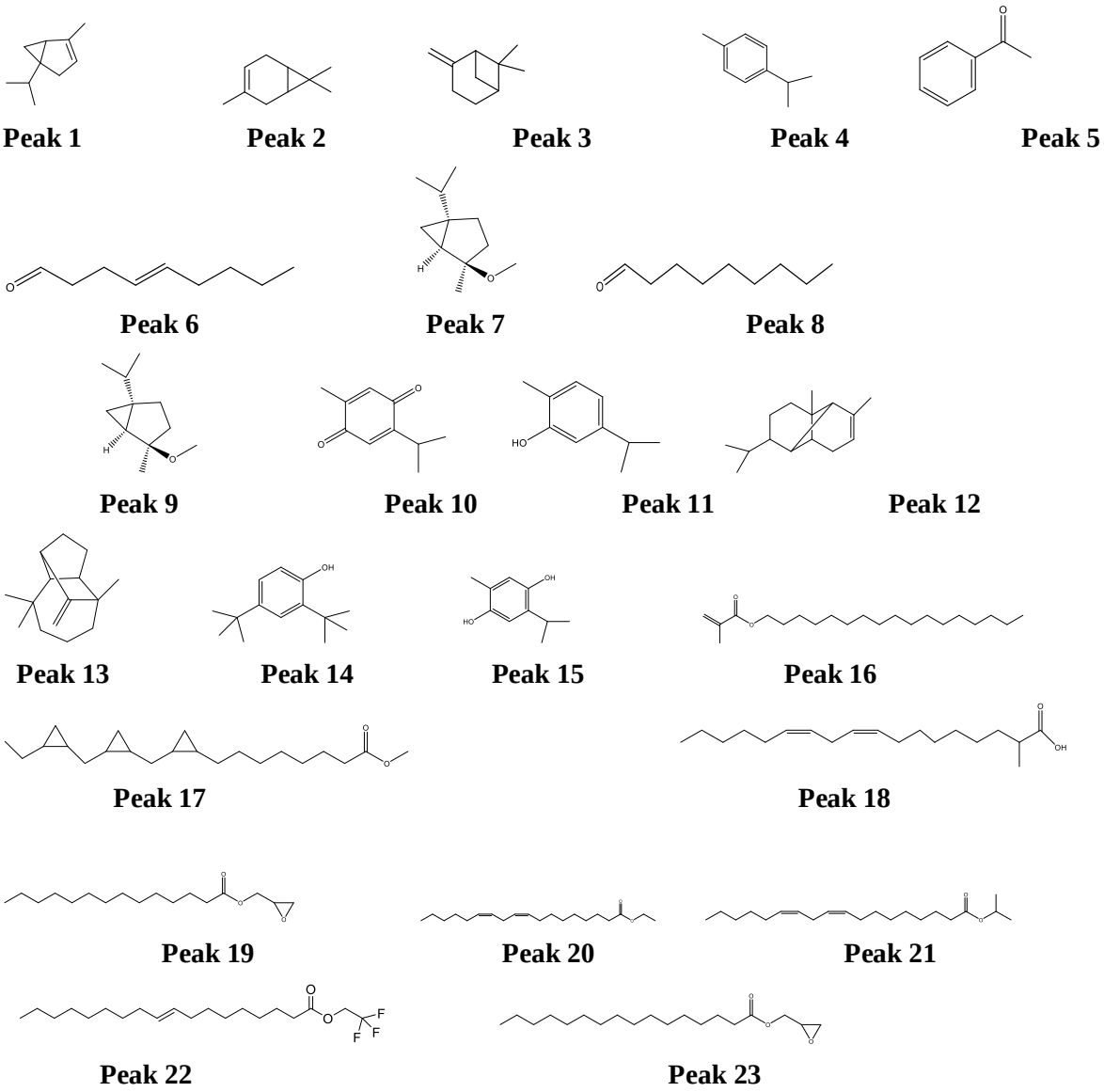
S. No.	Phytochemical	Area %	MS Fragment -ions
1.	n-Pentadecanol	31.56	29, 41, 55, 69, 88, 97, 111, 125, 140, 154, 182, 210
2.	9,12-Octadecadienoyl chloride	9.60	29, 41, 55, 67, 81, 95, 110, 121, 135, 149, 163, 262
3.	7-Hexadecenal	7.13	41, 55, 69, 81, 97, 109, 121, 135

Table 3: Phytochemicals discovered by GC-MS in the chloroform extract of the *Nigella sativa* seed sample:

Peak #	R.Time	Area%	Mol. weight	Mol. formula	Name of the compound
1	6.887	0.75	136	$C_{10}H_{16}$	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-
2	7.065	0.17	136	$C_{10}H_{16}$	3-Carene
3	8.248	0.24	136	$C_{10}H_{16}$	beta-Pinene
4	9.486	2.14	134	$C_{10}H_{14}$	p-Cymene
5	10.628	1.09	120	C_8H_8O	Acetophenone
6	11.320	0.15	140	$C_9H_{16}O$	4-Nonenal, (E)-
7	11.398	0.22	168	$C_{11}H_{20}O$	(1R,4R,5S)-1-Isopropyl-4-methoxy-4-methylbicyclo[3.1.0]hexane
8	11.605	0.32	142	$C_9H_{18}O$	Nonanal
9	12.019	0.32	168	$C_{11}H_{20}O$	(1R,4R,5S)-1-Isopropyl-4-methoxy-4-methyl[3.1.0]hexane
10	15.355	0.95	164	$C_{10}H_{12}O_2$	Thymoquinone
11	16.609	0.13	150	$C_{10}H_{14}O$	Phenol, 2-methyl-5-(1-methylethyl)-
12	17.616	0.14	204	$C_{15}H_{24}$	Ylangene
13	18.841	0.35	204	$C_{15}H_{24}$	Longifolene
14	20.528	0.21	206	$C_{14}H_{22}O$	2,4-Di-tert-butylphenol
15	21.481	0.83	166	$C_{10}H_{14}O_2$	p-Cymene-2,5-diol

16	27.598	0.46	324	$C_{21}H_{40}O_2$	Methacrylic acid, heptadecyl ester
17	27.960	2.76	334	$C_{22}H_{38}O_2$	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-,methyl ester
18	28.861	0.89	294	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid (Z,Z)-, methyl
19	28.966	2.13	284	$C_{17}H_{32}O_3$	Myristic acid glycidyl ester
20	29.670	26.31	308	$C_{20}H_{36}O_2$	Linoleic acid ethyl ester
21	30.010	39.15	322	$C_{31}H_{38}O_2$	Isopropyl linoleate
22	30.077	12.62	364	$C_{20}H_{35}F_3O_2$	9-octadecenoic acid, 2,2,2-trifluoroethyl ester
23	31.400	7.67	312	$C_{19}H_{36}O_3$	Glycidyl palmitate

Phytochemicals' structures were determined by GC-MS analysis of the *Nigella sativa* seed sample's chloroform extract**:**



Peak 1 **Peak 2** **Peak 3** **Peak 4** **Peak 5**

Peak 6 **Peak 7** **Peak 8**

Peak 9 **Peak 10** **Peak 11** **Peak 12**

Peak 13 **Peak 14** **Peak 15** **Peak 16**

Peak 17 **Peak 18**

Peak 19 **Peak 20** **Peak 21**

Peak 22 **Peak 23**

Table 4: Significant phytochemicals found in *Nigella sativa* seed extract in chloroform:

S. No.	Name	Area%	MS Fragment –ions
1.	Isopropyl linoleate	39.15	27, 41, 67, 81, 95, 109, 123, 137, 149, 263, 279
2.	Linoleic acid ethyl ester	26.31	29, 41, 55, 67, 81, 95, 109, 123, 135, 150, 164, 178, 220, 263
3.	9-octadecenoic acid, 2,2,2-trifluoroethyl ester	12.62	41, 55, 69, 83, 97, 111, 125, 139, 155, 207, 222, 265

Table 5: Phytochemicals discovered by GC-MS in the acetone extract of the *Nigella sativa* seed sample:

Peak#	R.Time	Area%	Mol. weight	Mol. Formula	Name of compound
1	6.887	0.97	136	$C_{10}H_{16}$	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-
2	7.949	1.43	174	$C_9H_{22}OSi$	1-Butyl(dimethyl)silyloxypropane
3	9.484	3.09	134	$C_{10}H_{14}$	p-Cymene
4	10.630	5.19	120	C_8H_8O	Acetophenone
5	11.600	0.35	142	$C_9H_{18}O$	Nonanal
6	12.021	0.54	168	$C_{11}H_{20}O$	(1R,4R,5S)-1-Isopropyl-4-methoxy-4-methylbicyclo[3.1.0]hexane
7	15.356	1.74	164	$C_{10}H_{12}O_2$	Thymoquinone
8	18.837	0.60	204	$C_{15}H_{24}$	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]-
9	20.531	0.67	206	$C_{14}H_{22}O$	2,4-Di-tert-butylphenol
10	21.479	4.49	166	$C_{10}H_{14}O_2$	p-Cymene-2,5-diol
11	26.695	2.57	270	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
12	27.164	0.52	278	$C_{16}H_{22}O_4$	Dibutyl phthalate
13	27.975	12.20	234	$C_{17}H_{30}$	1,8,11-Heptadecatriene, (Z,Z)-
14	28.862	7.71	294	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid (Z,Z)-, methyl
15	28.943	9.08	296	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester, (E)-
16	30.073	22.79	324	$C_{21}H_{40}O_2$	cis-9-Octadecenoic acid, propyl ester
17	31.389	26.06	312	$C_{19}H_{36}O_3$	Glycidyl palmitate

Phytochemicals discovered by GC-MS in the acetone extract of *Nigella sativa* seed sample:

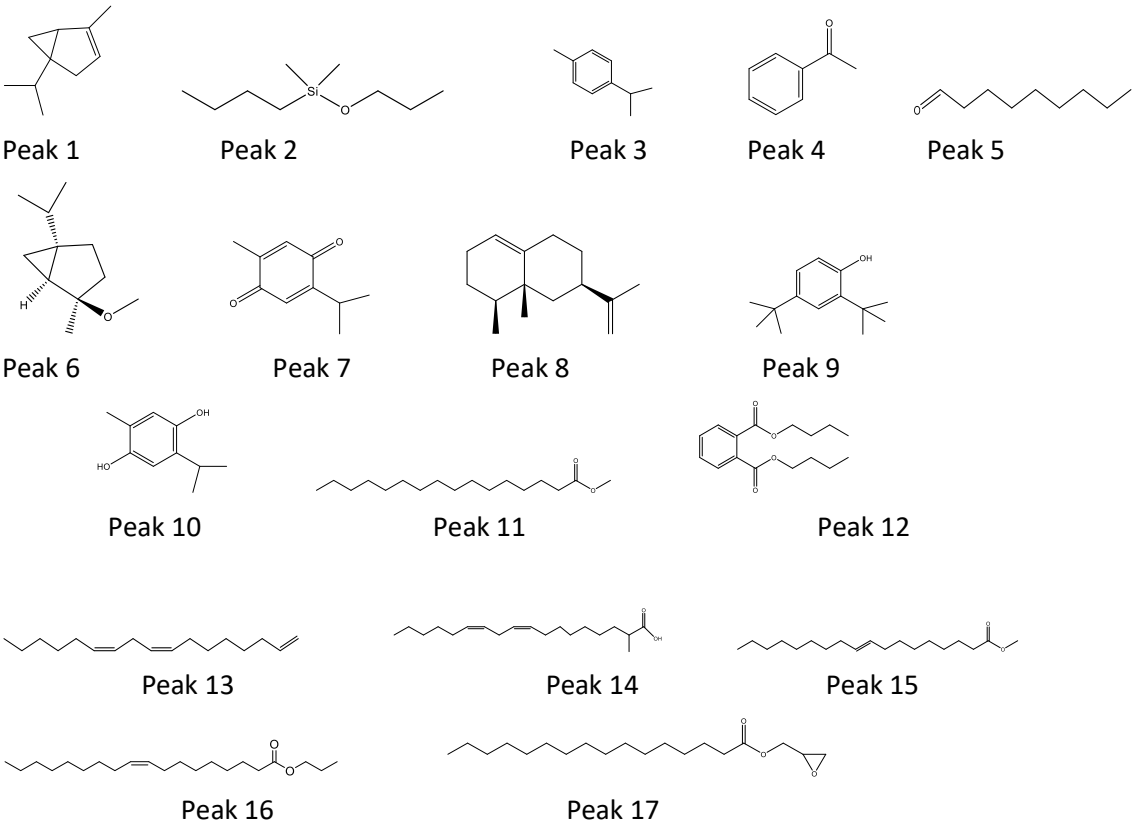


Table 6: Significant phytochemicals found in *Nigella sativa* seed extract in acetone:

S.N.	**Name**	**Area %**	**MS Fragment -ions**
1.	Glycidyl palmitate	26.06	29, 43, 57, 69, 84, 98, 112, 116, 129, 143, 154, 171, 185, 239, 269
2.	cis-9-Octadecenoic acid, propyl ester	22.79	29, 43, 55, 69, 83, 97, 111, 123, 137, 151, 169, 180, 222, 265
3.	1,8,11-Heptadecatriene, (Z,Z)-	12.20	29, 41, 54, 67, 81, 95, 109, 121, 135, 149, 163, 234

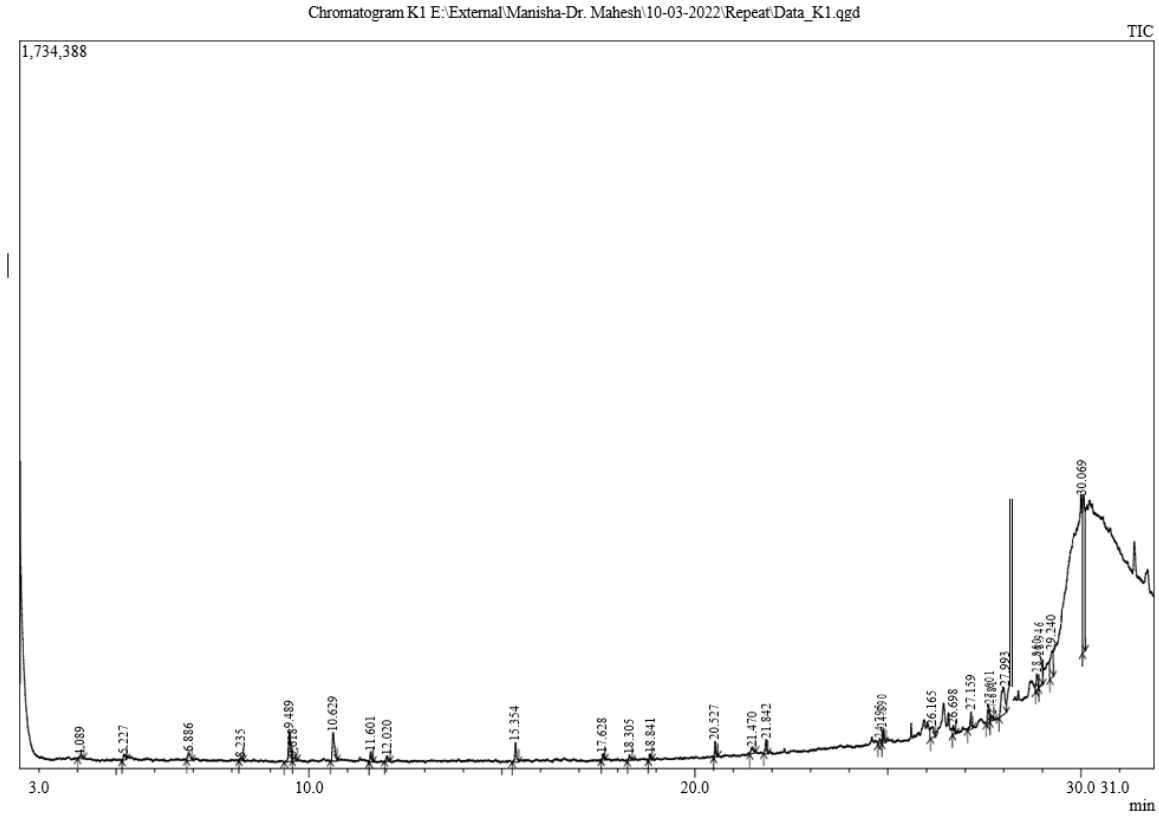


Figure 1: GC-MS Spectrum of Petroleum ether extract of *Nigella sativa* seeds.

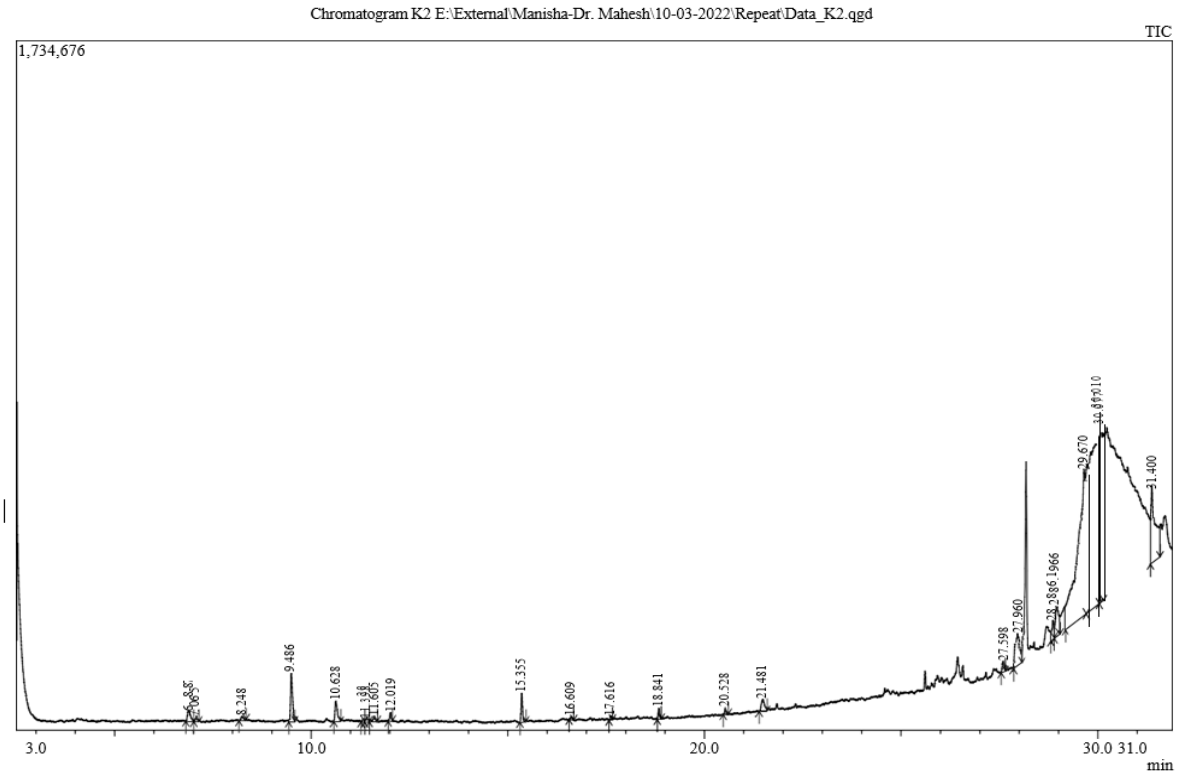


Figure 2: GC-MS Spectrum of chloroform extract of *Nigella sativa* seeds.

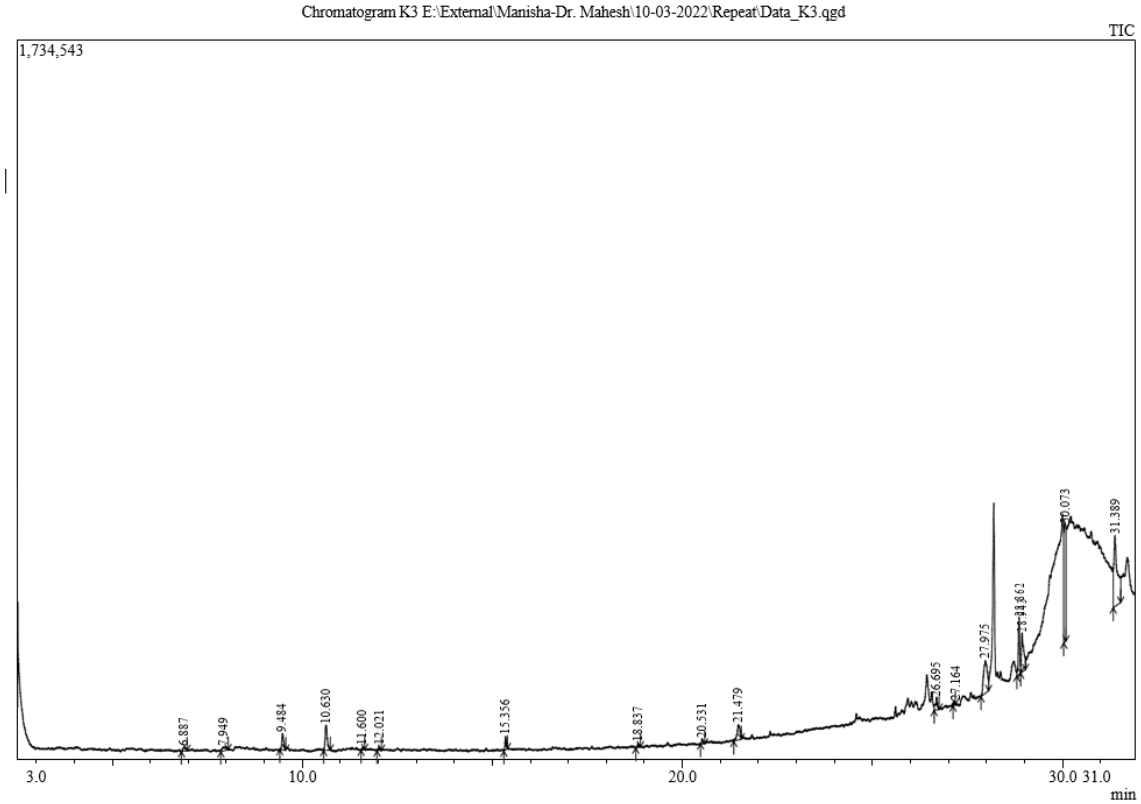


Figure 3: GC-MS Spectrum of acetone extract of *Nigella sativa* seeds.

Conflicts of interest

The authors declare no conflict of interest.

Author's contribution

Concept, collection, analysis of data, manuscript drafting and all experimental work like extraction and purification done by me in laboratory under the supervision of Dr. M.C. Sharma.

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References:-

1. Kokoskaa, L., Urbanovac, K., Kloucekd, P., Nedorostovad, L., Polesnaa, L., Malikb, J., Jirosc, P., Havlike, J., Vadlejchb, J., and Valterova, I., 2012. Essential Oils in the Ranunculaceae Family: Chemical Composition of Hydrodistilled Oils from Consolida regalis, Delphinium elatum, Nigella hispanica, and N. nigellastrum Seeds. *Chemistry & biodiversity*. 9, 151-161.
2. Ahmad, M.D.F., Ahmad, F.A., Ashraf, S.A., Saad, H.H., Wahab, S., Khan, M.I., 2020. An updated knowledge of Black seed (Nigella sativa Linn): Review of phytochemical constituents and pharmacological properties. *Journal of Herbal Medicine*. 1-47.
3. Mehta, B.K., Mehta, P., Gupta, M., 2009. A new naturally acetylated triterpene saponin from Nigella sativa. *Carbohydrate Research*. 344, 149–151.
4. Taskin M.K., Caliskan, O.A., Anil, H., Abougazar, H., Khan, I.A., Bedir, E., 2005. Triterpene Saponins from Nigella sativa L. *Turk J Chem*. 29 (5), 561 – 569.
5. Thakur, S., Kaurav, H., Chaudhary, G., 2021. Nigella sativa (Kalonji): A Black Seed of Miracle. *International Journal of Research and Review*. 8(4), 342-357.

6. Forouzanfar, F., Bazzaz, B.S.F., Hosseinzadeh, H., 2014. Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. *Iran J Basic Med Sci*. 17, 929-938.

7. Elbandy, M., Kang, O., Kwon, D., and Rho, J., 2009. Two New Antiinflammatory Tnterpene Saponins from the Egyptian Medicinal Food Black Cumin (Seeds of Nigella sativa). *Bull. Korean Chem. Soc.* 30(8), 1811-1816.

8. Javed, S., Shahid, A.A., Haider, M.S., Umeera, A., Ahmad, R., and Mushta, S., 2012. Nutritional, phytochemical potential and pharmacological evaluation of Nigella Sativa (Kalonji) and Trachyspermum Ammi (Ajwain). *Journal of Medicinal Plants Research*. 6(5), 768-775.

9. Zafar, K., Noorul, H., Nesar, A., Vartika, S., Khalid, M., Prashant, S., Zeeshan, A., Zohrameena, S., 2016. Pharmacological Activity of Nigella Sativa: A Review. *World J Pharm Sci*. 4(5), 234-241.

10. Dalli, M., Bekkouch, O., Azizi, S., Azghar, A., Gseyra N., and Kim B., 2022. Nigella sativa L. Phytochemistry and Pharmacological Activities: A Review (2019–2021). *Biomolecules*. 12(1), 20.

11. Parveen, A., Farooq, M.A., and Kyunn, W.W., 2020. A New Oleanane Type Saponin from the Aerial Parts of Nigella sativa with Anti-Oxidant and Anti-Diabetic Potential. *Molecules*, 25(9), 2171.

12. Desai, S.D., Saheb, S.H., Das, K.K., Haseena, S., 2015. Phytochemical Analysis of Nigella sativa and it`s Antidiabetic Effect. *J. Pharm. Sci. & Res.* 7(8), 527-532.

13. Kazemi M., 2014. Phytochemical Composition, Antioxidant, Anti-inflammatory and Antimicrobial Activity of Nigella sativa L. Essential Oil. *Journal of Essential Oil Bearing Plants*. 17(5), 1002-1011.

14. Ahmad, A., Husain, A., Mujeeb, M., Khan, S., Najmi, A.K., Siddique, N.A., Damanhouri, Z.A., Anwar, F., 2013. A review on therapeutic potential of Nigella sativa: A miracle herb. *Asian Pac J Trop Biomed*. 3(5), 337-352.

15. Khan, M.A., 1999. Chemical composition and medicinal properties of Nigella sativa Linn. *Inflammopharmacology*. 7(1), 15-35.

16. Talebi, A., Maham, M., Asri-Rezaei, S., Pournaghi, P., Khorrami, M.,and Derakhshan, A., 2021. Effects of Nigella sativa on Performance, Blood Profiles, and Antibody Titer against Newcastle Disease in Broilers. *Evidence-Based Complementary and Alternative Medicine*. 2021, 15.

17. Venkatachallam, S.K.T., Pattekhan, H., Divakar S., & Kadimi, U.S., 2010. Chemical composition of Nigella sativa L. seed extracts obtained by supercritical carbon dioxide. *J Food Sci Technol*. 47(6), 598–605.

18. Kabir, Y., Akasaka-Hashimoto, Y., Kubot, K., Komai, M., 2020. Volatile compounds of black cumin (Nigella sativa L.) seeds cultivated in Bangladesh and India. *Heliyon*. 6, e05343.

19. Khazdair, M.R., Ghafari, S., & Sadeghi, M., 2021. Possible therapeutic effects of Nigella sativa and its thymoquinone on COVID-19. *Pharmaceutical Biology*, 59(1), 696-703.

20. Tiji, S., Benayad, O., Berrabah, M., Mounsi, I. El., and Mimouni, M., 2021. Phytochemical Profile and Antioxidant Activity of Nigella sativa L Growing in Morocco. *Scientific World Journal*. 2021, 6623609, 12.

21. Islam, M.T., Khan, Md. R., Mishra, S.K., 2019. An updated literature-based review: phytochemistry, pharmacology and therapeutic promises of Nigella sativa L. *Oriental Pharmacy and Experimental Medicine*. 19, 115–129.