Solvent selection for efficient extraction, GC-MS and FT-IR characterization of major bioactive compounds present in different seed extracts of *Nigella sativa* L.

Nita Kaushik*[1], Aradhita Barmanray[2]

[1*] *Department of Food Technology, Guru Jambheshwar University of Science & Technology, Hisar-125001, Haryana, India*

[2] *Department of Food Technology, Guru Jambheshwar University of Science & Technology, Hisar-125001, Haryana, India*

Corresponding Author: Nita Kaushik

nitakaushikomg1996@gmail.com

Abstract

Objective: The present study was dealt with a rapid and accurate strategy for selection of various solvents and their combinations to obtain the efficient extraction of major bioactive compounds from different seed extracts of nigella sativa. GC-MS and FT-IR characterization is used for the identification and separation of major bioactive compounds in black cumin seed extracts.

Methods: Phytochemical screening was done for identifying and analyzing the presence of bioactive compounds through various chemical tests. To improve the extraction efficiency, six different solvents as methanol, acetone, ethanol, petroleum ether, ethyl acetate and distilled water were used in preparation of black cumin seed extracts. Methanol and acetone extracts showed the higher presence of phytochemicals as compare to other solvents. Further, six extracts of methanol and acetone extracts were prepared in combinations and the efficiency of all derived seed extracts were investigated by GC-MS and FTIR analysis.

Results: The findings of the study revealed that nigella seed extracts has potential phyto-constituents which triggers their biological activities. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *Nigella sativa* seed extracts revealed the existence of thymoquinone, t-butyl hydroquinone, o-cymene, thymol, longifolene, l-(+)-ascorbic acid, phenol, citronellal and many other bioactive compounds. The identification of phytochemicals is based on the peak area, retention time. The prepared strategy for various solvents selection provides a basis for further research on solvents selection for efficient extraction of phytochemicals from black cumin seed extracts.

Keywords: Seed extracts, Black cumin, Screening, Gas Chromatography-Mass Spectrometry, Phytochemicals and Extraction.

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1. Introduction

The annual herbaceous plant *Nigella sativa* L. of the Ranunculaceae family is also known as black caraway, black cumin, kalonji or kalajeera, or roman coriander (Kaushik and Barmanray, 2022). It is believed to have numerous health benefits, including antioxidant, anti-inflammatory and antimicrobial properties (Ardiana *et al.,* 2020). The major bioactive compounds of black cumin are thymoquinone, thymohydroquinone, thymol, p-cymene, carvacrol, α-pinene and β-pinene. Black cumin seeds have been used for centuries in traditional medicine and cuisine in the Middle East, Africa and Asia (Hameed *et al.,* 2015). Its dried seeds are a popular spice because of their peppery flavor, and they are used in a broad variety of traditional regional dishes around the world. Black seeds are valuable because of the volatile oil and bioactive substances, both of which have interesting chemical properties (Allah *et al.,* 2021). Before the development of modern medicine, the black seeds were utilized in traditional folk medicine to cure a wide variety of illnesses. Black seeds have a slightly bitter taste and a nutty, earthy flavor, and are often used to season bread, meat, and vegetable dishes (Hadi *et al.,* 2016). Overall, black cumin seeds are a versatile and nutritious ingredient with a long history of uses in traditional medicines and Indian cuisines (Lal *et al.,* 2020).

Black cumin seeds contain between 0.5% and 2.5% essential oils, which are responsible for their characteristic aroma and flavor (Jasim *et al.,* 2015). Nigella seeds are the store house of several bioactive compounds and the most abundant compounds in black cumin oil are thymoquinone (Solati *et al.,* 2014), p-cymene, carvacrol, α-thujene, thymol, and longifolene. Black cumin seeds contain about 20-25% protein, which is rich in essential amino acids like lysine, arginine, and methionine (Abdallah *et al.,* 2017). The black tiny seeds contain essential (0.40%-0.45%) and fixed (32-40%) oils, carbohydrates (31-33.9%), protein (16-20.85%), fibre (5.50%- 7.94%), tannins, alkaloids, saponins (Kinki *et al.,* 2020), minerals such as Fe, Ca, k, Mg, Zn and Cu (1.79%-3.44%), vitamin C, vitamin A, niacin, thiamine, pyridoxine. Other fatty acids found in black cumin seeds include palmitic, stearic, and eicosadienoic acids (Hangargekar *et al.,* 2020). Black cumin seeds were discovered in monuments and traditional medical writings recorded by significant civilizations including

the Greco-Roman and the Malaysian, and its advantages and nutritional and medicinal purposes are described in the Islamic religion, prophetic medicine, and other religions (Afoakwah and Mahunu, 2023). In addition to its aesthetic value, the nigella seeds can be used medicinally due to its antioxidant, hepatoprotective, anti-ulcer, antibacterial, anti-inflammatory, anticancer, analgesic, antidiabetic and other antimicrobial activities (Mariod, 2022).

Figure: 1 ***Nigella sativa*** **flower and seeds**

The extraction of bioactive compounds from natural sources, such as plants, herbs, or microorganisms, is a crucial step in various fields, including pharmaceuticals, food science, and natural products research. Bioactive compounds have specific biological activities and are important for their potential health benefits (Neel *et al.*, 2023). The last few years gas chromatography (GC-MS) has become firmly established as a key technological for secondary metabolites in plant extracts. GC-MS analysis of plant extracts is widely used in phytochemistry, pharmacognosy, and herbal medicine research to study the chemical constituents of plants, assess their medicinal properties, and ensure product quality and safety in the herbal and dietary supplement industry. It is a powerful tool for characterizing the chemical diversity of plants and their potential therapeutic applications (Nazeer and George, 2017). So, the present study was aimed to investigate the composition of major components in black cumin seed extracts, their identification along with separation by subjecting to GC-MS analysis. Fourier Transform Infrared Spectroscopy is also an analytical technique used to identify the functional groups present in plant extracts. The FTIR analysis method uses infrared light to scan test samples and used to observe their chemical properties.

The main goal of this study was to define the most suitable solvent for efficient extraction of major phytochemicals from black cumin seed extracts. Six different solvents were selected for the extraction: methanol, ethanol, acetone, petroleum ether, ethyl acetate and distilled water. Chemical composition of the obtained extracts was monitored by Gas chromatography–mass spectrometry and Fourier Transform Infrared Spectroscopy. Besides investigating the capacity of different solvents to extract specific constituents, the aim of this study was also to evaluate potential bioactive natural compounds and promote their utilization.

2. Material and Methods

2.1. Collection of Plant Material

The seeds of *Nigella sativa* L. were procured during the month of April, 2023 at properly matured (dark black colour) stage from National Research Centre on Seed Spices ICAR NRCSS, Tabiji, Ajmer, Rajasthan, India. NRCSS designated this study material as Ajmer Nigella (AN -1 Variety).

2.2. Preparation of Plant Materials Extraction

In order to remove any traces of dirt or other contaminants, the seeds were first washed thoroughly with tap water, and then washed again with distilled water. The washed black seeds (AN-1) were blotted on blotting paper and shade dried at room temperature. Fine powder of dried seeds was obtained by electric grinder for 1 minute until fine powder was obtained to further proceed with experiments followed by Reddy *et al.,* (2018).

2.3. Chemicals

All solvents were used of analytical grade. Methanol (99.85%), Ethanol (99.6%), Acetone (99.9%), Petroleum ether (99.85%), Ethyl Acetate (99.5%), distilled water (99.9%). Standards of bioactive compounds as thymoquinone and thymol were purchased from Sigma Aldrich.

2.4. Phytochemical Screening

The seed extracts were subjected to preliminary phytochemical investigations to determine the different phyto-constituents using standard methods followed by (Yessuf, 2015). Phytochemicals are secondary constituent gives plants with colour, flavour and perform various functions in plants, such as protection against insects and other predators, and

protection against environmental stressors like UV radiation and pollution (Saleh *et al.,* 2018).

3. Gas Chromatography and Mass Spectroscopy (GC-MS) Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis is a powerful analytical technique that is commonly used to identify and quantify the chemical components present in complex mixtures found in plant extracts (Nivetha and Prsasanna, 2016). GC-MS analysis of different seed extracts of black cumin were carried out using the equipment Gas Chromatography-Mass Spectrometry Shimadzu QP 2010 Ultra GC-MS, Japan. The GC equipped with a fused silica capillary column Rxi®-5 Sil MS with a dimension of 20m×0.18mm ID, 0.18µm film thickness. The diluted sample injected by normal mode and the helium gas flow rate consistently maintained at 1ml/min. The oven temperature programmed at 70ºC for 5 min. Mass Spectrometry conditions as electron energy with 70eV and electron impact (EI) ion source temperature 200ºC given in Table 2.

The relative percentage amount of each component was calculated by comparing its average peak area to the total mass. Interpretation on mass spectrum of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of test materials were ascertained and the results obtained have been tabulated.

4. Fourier Transform-Infrared Spectroscopy (FTIR) Analysis

Fourier-Transform Infrared Spectroscopy is a powerful analytical technique used to identify and characterize the chemical composition of materials based on their interaction with infrared light. Using an FTIR spectrometer, the compounds' spectra were captured, and the functional groups were identified by measuring the transmission percentage in proportion to wave number (cm). Sample concentrations were combined with 100 mg of potassium bromide (KBr) to create pellets, which were then used to gather spectra at wave numbers between 4000 and 600 cm^{-1}.

5. Results and Discussion

5.1. Preliminary Phytochemical Screening

The distribution of different phytochemicals in different seed extracts of *Nigella sativa* L. were evaluated quantitatively. As the results in table 1 specific tests for phytochemicals in *Nigella sativa* seed extracts in different solvents as methanol, ethanol, acetone, petroleum ether, ethyl acetate and distilled water contains alkaloids, saponins, terpenoids, steroids, tannins, flavonoids and phenolic compounds. The alkaloids, flavonoids, steroids and phenolic compounds are highly present in methanolic extracts of *Nigella sativa* while flavonoids are absent in petroleum ether and distilled water extracts. While acetone extract shows the higher presence of flavonoids. Petroleum ether and ethyl acetate extract shows the absence of phenolic compounds.

On the other hand, steroids are highly (+++) and alkaloids are moderately (++) present in petroleum ether extracts.

Table 1. Phytochemical Screening of *Nigella sativa* seed extracts

Phyto-constituents	NS.MT	NS.ET	NS.AC	NS.PE	NS.EA	NS.DW
Terpenoids	+	+	+	+	-	-
Saponins	+	+	-	-	-	+
Steroids	++	+	+	+++	+	+
Alkaloids	++	+	+	++	+	+
Phenols	++	++	+	-	-	+
Flavonoids	++	+	+++	-	+	-

Note: All the values expressed in the table are the mean of three replication. (+++: high, ++: moderate, +: present, -: not detected). Where (NS.MT: *Nigella sativa* methanol extract, NS.ET: *Nigella sativa* ethanol extract, NS.AC: *Nigella sativa* acetone extract, NS.PE: *Nigella sativa* petroleum ether extract, NS.EA: *Nigella sativa* ethyl acetate extract, NS.DW: *Nigella sativa* distilled water extract)

Table 2. Operation conditions for GC-MS analysis of black cumin seed extracts

Column	GC-MS(20m×0.18mm,0.18µmfilm thickness)
Injection	Split less
Injector Temperature	250ºC
Carrier Gas flow	1ml/min

Detector Temperature	200ºC
Oven Temperature	70ºC for 5 minutes 310ºC for 10 minutes

5.2. Gas Chromatography-Mass Spectrometry (GC-MS Analysis)

The results pertaining to GC-MS analysis led to the identification of number of compounds from various extracts from different solvents. GC-MS chromatogram showed many peaks, indicating the presence of various compounds in the given tables below. Many of the compounds reported through GC-MS in the study are first time identified with the respective solvents and are found highest amount even through preliminary phytochemical studies.

In the present study, Gas Chromatography Mass Spectrometry analysis was carried out in six single solvent extracts (methanol, ethanol, acetone, petroleum ether, ethyl acetate, distilled water) of nigella seeds in **figure 2** and six extracts of two solvent mixtures (methanol and acetone with water) as methanol: water (25:75), methanol :water (50:50), methanol: water (75:25) and acetone: water (25:75), acetone: water (50:50), acetone: water (75:25) shown in **figure 3.**

Table 3. GC-MS Analysis of Methanolic Extract of *Nigella sativa*

Sr. No.	Name of the compound	Retention Time (t)	Area Percentage (%)
1.	p-Cymene-7-ol	17.109	49.64
2.	1-Cyclohexene-1-acetaldehyde	24.418	22.81
3.	Thymoquinone	15.621	14.70
4.	Linoleic acid ethyl ester	37.198	2.78
5.	2-Napthealenemethanol	27.673	2.14
6.	9,12-Octadecenoic acid, methyl ester	35.917	2.05
7.	9-Octadecenoic acid, methyl ester	36.049	1.14
8.	Ethyl Oleate	37.322	1.12
9.	Hexadecanoic acid	32.545	0.78
10.	Hexadecanoic acid, ethyl ester	33.939	0.59
11.	13-Docosenoic acid	43.213	0.38
12.	Cyclopropaneoctanoic acid	43.322	0.32
13.	Methyl stearate	36.560	0.29
14.	Linoleic acid, ethyl ester	47.639	0.21
15.	Dimantine	50.696	0.15
16.	Linoleic acid, ethyl ester	40.437	0.15
17.	1,3,6,10-Cyclotetradecatetraene	44.186	0.13
18.	Docosaenoic acid, methyl ester	43.632	0.12
19.	9,12- Octadecanoic acid,methyl ester	39.671	0.11

20.	9-Octadecenoic acid, methyl ester	36.164	0.11
21.	Benzenepropanoic acid	32.655	0.11
22.	9,12-Octadecenoic acid, methyl ester	40.837	0.10
23.	9,12- Octadecanoic acid,methyl ester	37.822	0.09

Twenty three phytochemicals were identified in the methanolic extract of nigella sativa as shown in table 3. P-Cymene-7-ol and thymoquinone are the major phytochemicals of black seeds having the higher area percentage as 49.64% and 14.70%.

Table 4. GC-MS Analysis of Ethanolic Extract of *Nigella sativa*

Sr. No.	**Name of the compound**	**Retention Time (t)**	**Area Percentage (%)**
1.	Neopentyl alcohol, TMS derivative	10.714	29.70
2.	Linoleic acid, methyl ester	37.233	23.01
3.	Ethyl acetate	9.574	14.67
4.	Ethyl Oleate	37.352	13.25
5.	Hexadecanoic acid, ethyl ester	33.980	6.93
6.	Linoleic acid, ethyl ester	40.858	1.48
7.	Octadecanoic acid, ethyl ester	37.847	1.47
8.	Decanoic acid, ethyl ester	19.877	1.41
9.	Hexadecanoic acid	43.448	1.15
10.	Octanoic acid, ethyl ester	13.967	1.13
11.	Tetradecane	20.043	0.53
12.	9,12-Octadecanoic acid	35.952	0.48
13.	Hexadecane	25.263	0.48
14.	3,3-Diethoxy-1-propanol	21.589	0.47
15.	Trisphosphate	59.683	0.46
16.	Dodecanoic acid, ethyl ester	25.097	0.43
17.	9- Octanoic acid, ethyl ester	36.084	0.33
18.	Cis,cis-7,10-Hexadecadienal	40.463	0.23
19.	Ethyl 9-hexadecanoate	40.957	0.22
20.	Pentadecane	22.740	0.22
21.	Hexadecanoic acid	43.717	0.21
22.	1-Monopalmitin, 2TMS derivative	42.742	0.20
23.	Pentadecanoic acid, 3-methylbutyl ester	26.334	0.18
24.	Phenol, 2,4-Bis(2-dimethylethyl) ester	56.219	0.16
25.	Decanedioic acid, bis(2-ethylhexyl) ester	40.712	0.14
26.	Hexadecanoic acid, methyl ester	32.605	0.13
27.	1,2-benezenedicarboxylic acid	31.218	0.13
28.	Octadecane	29.925	0.12
29	Decanedioic acid	47.757	0.12
30.	Ethyl9-hexadecenoate	33.549	0.10

31.	Pentanoic acid, 4-methyl-ethyl ester	29.743	0.10
32.	Heneicosane	27.655	0.10
33.	Ehtyl,3,3-diethoxypropionate	24.912	0.08
34.	Eciosanoic acid, ethyl ester	41.412	0.07
35.	Propane,1,1,3,3-tetraethoxy-Malonaldehyde	26.061	0.07

Thirty five phytochemicals were identified in the ethanolic extract of nigella sativa as shown in table 4. Neopentyl alcohol, TMS derivative, Linoleic acid, methyl ester are the major phytochemicals of black seeds having the higher area percentage as 29.70% and 23.01%.

Table 5. GC-MS Analysis of Acetone Extract of *Nigella sativa*

Sr. No.	**Name of the compound**	**Retention Time (t)**	**Area (%)**
1.	Phenol, 2,3,5,6-tetramethyl-Durenol	17.038	38.99
2.	Linoleic acid	37.215	12.88
3.	t-butylhydroquinone	24.382	8.29
4.	Thymoquinone	15.609	8.19
5.	Ethyl oleate	37.331	5.46
6.	9,12-Octadecadienoic acid	35.929	5.45
7.	13-Docosenoic acid, methyl ester	43.234	4.75
8.	9-Octadecenoic acid, methyl ester	36.061	3.46
9.	13-Docosenoic acid, methyl ester	43.333	2.91
10.	Hexadecanoic acid, ethyl ester	33.951	1.92
11.	Hexadecanoic acid, methyl ester	32.558	1.26
12.	Docosanoic acid, methyl ester	43.647	0.96
13.	6,9-Octadecadienoic acid, methyl ester	47.682	0.82
14.	Linoleic acid, ethyl ester	40.843	0.57
15.	Methyl stearate	36.575	0.52
16.	Octadecanoic acid,	37.830	0.45
17.	11, Eciosenoic acid	39.788	0.44
18.	9-Octadecenoic acid	36.182	0.43
19.	9,12-Octadecadienoly chloride	47.733	0.39
20.	Tetracosanoic acid	46.785	0.39
21.	9,12-Octadecenoic acid	36.691	0.31
22.	Eciosanoic acid	40.252	0.27
23.	11- Eciosanoic acid	30.902	0.24
24.	Erucic acid	45.607	0.23
25.	9-Octadecenoic acid	46.424	0.22
26.	2-Hydroxymethyl palmitate, TMS derivative	45.023	0.22

Twenty six phytochemicals were identified in the acetone extract of nigella sativa as shown in table 5. Phenol, Linoleic acid, t-butyl hydroquinone and thymoquinone are the major

phytochemicals of black seeds having the higher area percentage as 38.99%, 12.88%, 8.29%, and 8.19%.

Table 6. GC-MS Analysis of Petroleum Ether Extract of *Nigella sativa*

Sr. No.	Name of the compound	Retention Time (t)	Area Percentage (%)
1.	Oleic acid	37.531	31.79
2.	1-Ascorbic acid	34.827	30.28
3.	9-Octadecenoic acid	39.492	18.27
4.	Hexadecanoic acid	34.041	3.88
5.	1-Cyclohexene-1-acetaldehyde	24.310	2.49
6.	6,9- Octadecenoic acid	36.054	2.22
7.	9-Octadecenoic acid, methyl ester	36.186	1.57
8.	Sorbitol	15.617	1.41
9.	2-Propenoic acid	45.653	1.16
10.	o-Cymene	8.315	1.14
11.	Isopropyl linoleate	41.134	1.06
12.	Phenanthrene	33.276	0.46
13.	Tetradecanoic acid	29.285	0.40
14.	D-Mannitol	10.805	0.40
15.	Isopropyl linoleate	57.602	0.32
16.	Longifolene	20.403	0.29
17.	2,3-Dihydro farnesyl acetate	55.324	0.26
18.	Hexadecanoic acid	32.589	0.26
19.	Cyclohexanol	11.447	0.24
20.	Silane	18.354	0.22
21.	Tris-phosphate	59.716	0.20
22.	1,3-Dipalmitin, TMS Derivative	43.026	0.20
23.	9-Octadecenoic acid, methyl ester	47.689	0.17
24.	Carbamic acid	42.744	0.16
25.	2H-Benzopyran-6-ol	52.753	0.14
26.	Thymol	17.273	0.14
27.	Heneicosane	22.399	0.12
28.	Squalene	48.082	0.11
29.	7,9-Di-tert-butyl-oxaspiro	32.206	0.11
30.	Dotriacontane	27.626	0.11
31.	Hexadecane	16.401	0.08
32.	Heptadecane	22.600	0.07
33.	Tricycloundec-9-ene	27.523	0.06
34.	Heneicosane	18.702	0.06
35.	Hexadecane	22.293	0.05
36.	Hexadecane	23.580	0.04
37.	Phenol	22.963	0.04

38.	Dotriacontane	30.693	0.02

Thirty eight phytochemicals were identified in the petroleum ether extract of nigella sativa as shown in table 6. Oleic acid, octadecanoic acid, o-cymene, longifolene and thymol are the major phytochemicals of black seeds having the higher area percentage as 31.79%, 18.27%, 1.14%, and 0.14%.

Table 7. GC-MS Analysis of Ethyl Acetate Extract of *Nigella sativa*

Sr. No.	Name of the compound	Retention Time (t)	Area Percentage (%)
1.	2H-2,4a-Methanonapthalen	37.556	29.51
2.	Linoleic acid ethyl ester	36.052	10.19
3.	Hexadecanoic acid, propyl ester	34.201	9.65
4.	n- Hexadecanoic acid	34.848	8.68
5.	Ethyl Oleate	41.154	8.08
6.	Isopropyl linoleate	47.824	5.80
7.	Ethyl 9-Hexadecenoate	35.557	5.55
8.	Undecanal, 2-methyl-Aldehyde	42.610	3.91
9.	9,12-Octadecadienoic acid	46.402	2.80
10.	2-Deoxy-D-Glucose	18.807	2.20
11.	Hexadecanoic acid	44.931	2.15
12.	9-Octadecenoic acid	38.883	1.98
13.	Isopropyl linoleate	41.908	1.81
14.	9,12-Octadecadienoic acid	48.470	1.64
15.	Glycerol 2-acetate 1,3-dipalmitate	45.797	1.44
16.	t-Butylhydroquinone	24.587	1.02
17.	Ethyl 9-hexadecenoate	33.562	0.93
18.	Cis,cis-7,10-hexadecadienal	44.210	0.79
19.	Tetradecanoic acid	29.770	0.44
20.	Hexadecanoic acid, methyl ester	32.606	0.38
21.	6,9-Octadecadienoic acid	50.592	0.28
22.	Isopropyl linoleate	57.609	0.15
23.	Alpha-D-Galactopyranoside	17.473	0.13
24.	Phenanthrene	33.282	0.11
25.	Stigmasterol	55.274	0.09
26.	Pentadecanoic acid	31.924	0.07
27.	Longifolene	20.427	0.07
28.	2H-1-Benzopyran-6-ol	52.768	0.05
29.	9-Octadecenoic acid, ethyl ester	31.294	0.04
30.	Dotriacontane	27.630	0.02
31.	Heneicosane	22.406	0.02
32.	Ethyl Oleate	29.110	0.01
33.	1-Napthalenol	27.244	0.01
34.	Hexadecane	25.270	0.01

Thirty four phytochemicals were identified in the ethyl acetate extract of nigella sativa as shown in table 7. 2H-2,4a-Methanonapthalen, linoleic acid and hexadecanoic acid are the major phytochemicals of black seeds having the higher area percentage as 29.51%, 10.19%, and 9.65%.

Table 8. GC-MS Analysis of Aqueous Extract of *Nigella sativa*

Sr. No.	Name of the compound	Retention Time (t)	Area Percentage (%)
1.	Linoleic acid	37.328	34.83
2.	Ethyl oleate	37.439	20.04
3.	6,9-Octadecadienoic acid	46.248	11.51
4.	Hexadecanoic acid	34.012	11.17
5.	Linloeic acid	40.864	3.23
6.	Hexadecanoic acid	43.407	2.54
7.	Ethanethioamide	10.974	2.31
8.	1,3-Propanediol	22.304	2.18
9.	Trilinolein	52.260	1.74
10.	Octadecanoic acid	37.863	1.81
11.	Octadecanoic acid	46.658	1.25
12.	Linoleic acid	35.947	1.08
13.	9,12-Octadecadienoic acid	55.062	1.05
14.	9,12-Octadecadienoic acid	40.452	1.01
15.	9-Octadecanoic acid	36.080	0.55
16.	Stigmasterol	54.303	0.50
17.	Trisphosphate	59.693	0.30
18.	1,3,6,10-Cyclotetradecatetraene	44.162	0.24
19.	Ethyl 9-hexadecenoate	33.534	0.23
20.	Hexadecanoic acid	32.584	0.20
21.	t-Butylhydroquinone	24.378	0.20
22.	Octadecanamide	40.130	0.18
23.	Propane	15.197	0.18
24.	9-Octadecanoic acid	42.754	0.17
25.	Eciosanoic acid	41.395	0.17
26.	7,9-Di-tertbutyl-oxaspiro	32.197	0.17
27.	9,12-Octadecadienoic acid	42.667	0.14
28.	Tetradecanoic acid	29.743	0.14
29.	Pyrrolidine	40.011	0.12
30.	Isopropyl linoleate	39.697	0.12
31.	Dibutyl pthalate	33.213	0.12
32.	Carbamic acid	42.391	0.09
33.	1H-Benzocyclohepten-7-ol	41.492	0.09

34.	1,2-Cyclopentanediol	18.931	0.09
35.	1,2-Benzenedicarboxylic acid	31.207	0.08
36.	3-Cyclohexene-1-methanol	23.669	0.05
37.	6,9-Octadecadienoic acid	39.279	0.04
38.	Pentadecanoic acid	31.909	0.04
39.	Gamma-Dodecalactone	26.583	0.04

Thirty nine phytochemicals were identified in the aqueous extract of nigella sativa as shown in table 8. Linoleic acid, ethyl oleate, 6, 9-Octadecadienoic acid are the major phytochemicals of black seeds having the higher area percentage as 34.83%, 20.04% and 11.51%.

Table 9. GC-MS analysis of acetone: water (25:75) solvent extract of *Nigella sativa* seeds

Sr. No.	Name of the compound	Retention Time (t)	Area (%)
1.	13-Docosenoic acid	43.231	36.02
2.	13-Docosenoic acid, methyl ester	43.332	21.36
3.	Docosanoic acid	43.642	6.99
4.	Diethyl pthalate	24.984	5.32
5.	9-Octadecenoic acid	36.069	4.53
6.	15-Tetracosenoic acid	46.418	3.95
7.	9-Octadecenoic acid	36.189	3.73
8.	11-Eciosenoic acid	39.783	2.76
9.	Tetracosanoic acid	46.795	2.71
10.	Oleic acid, butyl ester	45.607	2.59
11.	9,11-Octadecadienoic acid	35.927	2.20
12.	Hexadecanoic acid	32.563	2.13
13.	Methyl stearate	36.574	2.11
14.	9-Octadecenoic acid	39.904	1.97
15.	Eciosaenoic acid	40.256	1.63

Fifteen phytochemicals were identified in the acetone: water (25:75) solvent extract of nigella sativa as shown in table 9. 13-Docosenoic acid, docosanoic acid and diethyl pthalate are the major phytochemicals of black seeds having the higher area percentage as 36.02%, 6.99% and 5.32%.

Table 10. GC-MS analysis of acetone: water (50:50) solvent extract of *Nigella sativa* seeds

Sr. No.	Name of the compound	Retention Time (t)	Area (%)
1.	13-Docosenoic acid	43.228	30.86
2.	13-Docosenoic acid, methyl ester	43.332	18.64
3.	13-Docosenamide	47.590	11.74
4.	Docosanoic acid	43.646	6.10
5.	9-Octadecenoic acid	36.061	5.87
6.	9,12-Octadecadienoic acid	35.928	4.24

7.	Hexadecanoic acid	32.560	3.93
8.	Behenic alcohol	45.607	3.42
9.	11-Eicosenoic acid	39.791	3.02
10.	Eciosanoic acid	40.256	2.31
11.	Tetracosanoic acid	46.782	2.26
12.	9-Octadecenoic acid	36.191	2.20
13.	15-Tetracosenoic acid	46.412	1.93
14.	Methyl stearate	36.568	1.83
15.	11-Eicosenoic acid	39.896	1.65

Fifteen phytochemicals were identified in the acetone: water (50:50) solvent mixture extract of nigella sativa as shown in table 10. 13-Docosenoic acid, docosenamide are the major phytochemicals of black seeds having the higher area percentage as 30.86% and 11.74%.

Table 11: GC-MS analysis of acetone: water (75:25) solvent extract of *Nigella sativa* seeds

Sr. No.	Name of the compound	Retention Time (t)	Area (%)
1.	Isopropyl linoleate	37.872	96.34
2.	n-Hexadecanoic acid	33.781	1.03
3.	9,12-Octadecadienoic acid	35.926	0.99
4.	9-Octadecenoic acid	36.060	0.66
5.	t-Butylhydroquinone	24.405	0.23
6.	9-Octadecenoic acid	45.611	0.19
7.	Hexadecanoic acid, methyl ester	32.541	0.15
8.	1,3-Dipalmitin, TMS derivative	42.782	0.13
9.	13-Docosenoic acid	43.255	0.12
10.	Hexanedioic acid	41.329	0.10
11.	Citronellal	30.184	0.04

Eleven phytochemicals were identified in the acetone: water (75:25) solvent mixture extract of nigella sativa as shown in table 11. Isopropyl linoleate, n-Hexadecanoic acid are the major phytochemicals of black seeds having the higher area percentage as 96.34%, 1.03%.

Table 12. GC-MS analysis of methanol: water (25:75) solvent extract of *Nigella sativa*

Sr. No.	Name of the compound	Retention Time (t)	Area (%)
1.	Alpha-D-Galactopyranoside	25.456	72.14
2.	Thymol	17.098	6.22
3.	Hexadecanoic acid	43.429	5.42
4.	13-Docosenoic acid	43.211	5.05
5.	13-Docosenoic acid	43.313	3.93
6.	Dimantine	50.706	2.00
7.	Docosanoic acid	43.635	1.75

8.	1,2-Benzenedicarboxylic acid	31.183	1.11
9.	2,4-Di-tert-butylphenol	22.957	0.94
10.	Hexadecanoic acid	32.549	0.76
11.	9-Octadecanoic acid	36.045	0.68

Eleven phytochemicals were identified in the methanol: water (25:75) solvent mixture extract of nigella sativa as shown in table 12. Alpha-D-Galactopyranoside, thymol and hexadecanoic acid are the major phytochemicals of black seeds having the higher area percentage as 72.14%, 6.22% and 5.42%.

Table 13. GC-MS analysis of methanol: water (50:50) solvent extract of *Nigella sativa*

Sr. No.	**Name of the compound**	**Retention Time (t)**	Area **(%)**
1.	Alpha-D-Galactopyranoside	25.743	87.28
2.	β-D-Ribopyranoside	18.750	2.52
3.	t-Butylhydroquinone	24.514	2.15
4.	5,12-Napthacenedione	38.990	1.37
5.	Hexadecanoic acid	43.419	1.14
6.	13-Docosenoic acid	43.212	1.07
7.	Phenol	17.084	0.81
8.	13-Docosenoic acid	43.318	0.76
9.	Androstane-3,17-dione	44.182	0.75
10.	Dothiepin	50.709	0.57
11.	2,4-Di-tert-butylphenol	22.955	0.41
12.	1,2-Benzenedicarboxylic acid	31.184	0.36
13.	4-Phenoxyacetophenone	29.294	0.36
14.	9-Octadecenoic acid	36.049	0.29
15.	Hexadecanoic acid	32.544	0.14

Fifteen phytochemicals were identified in the methanol: water (50:50) solvent mixture extract of nigella sativa as shown in table 13. Alpha-D-Galactopyranoside, β-D-Ribopyranoside, t-butyl hydroquinone are the major phytochemicals of black seeds having the higher area percentage as 87.28%, 2.52% and 2.15%.

Table 14. GC-MS analysis of methanol: water (75:25) solvent extract of *Nigella sativa*

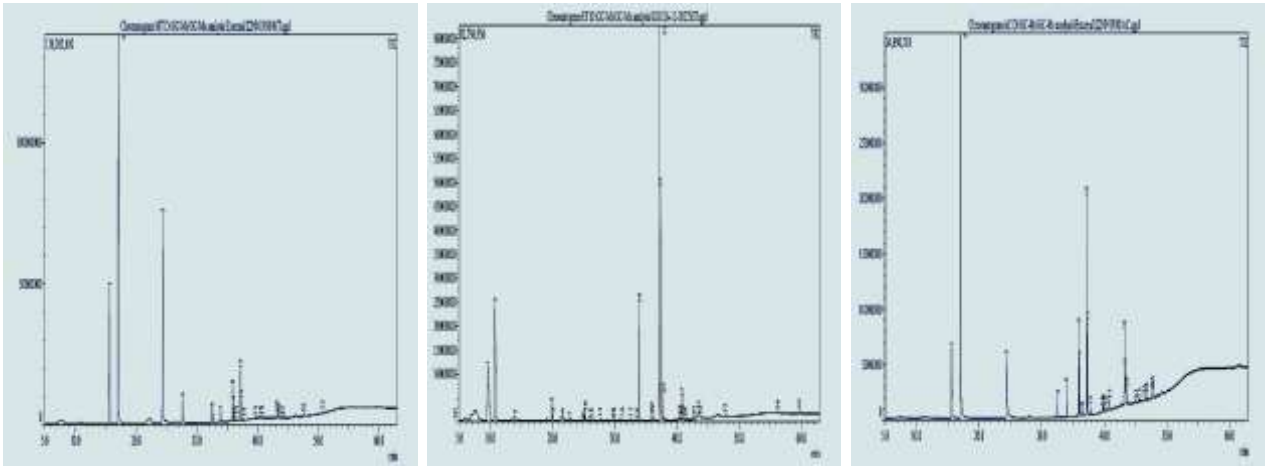
Sr. No.	**Name of the compound**	**Retention Time (t)**	**Area (%)**
1.	Alpha-D-Galactopyranoside	26.297	53.21
2.	9-Octadecenoic acid	39.634	12.87
3.	9,12-Octadecanoic acid	46.246	7.58
4.	9,12-Octadecanoic acid	35.918	4.03
5.	t-Butylhydroquinone	24.390	3.96
6.	Hexadecanoic acid	43.430	3.41
7.			

8.	13-Octadecenal	36.048	2.80
9.	Citronellal	30.183	2.42
10.	9-Octadecenoic acid	39.261	1.94
11.	11,14,17-Eicosatrienoic acid	40.263	1.50
12.	Ledol	44.186	1.31
13.	Phenol	17.296	1.27
14.	13-Docosenoic acid	43.222	0.70
15.	n-Hexadecanoic acid	33.422	0.70
16.	Citronellal	28.458	0.58
17.	Oxiraneoctanoic acid	39.840	0.50
18.	Oxiraneoctanoic acid	40.524	0.48
19.	Cyclopropanecarboxylic acid	41.524	0.42
20.	9-Octadecenoic acid	38.980	0.31

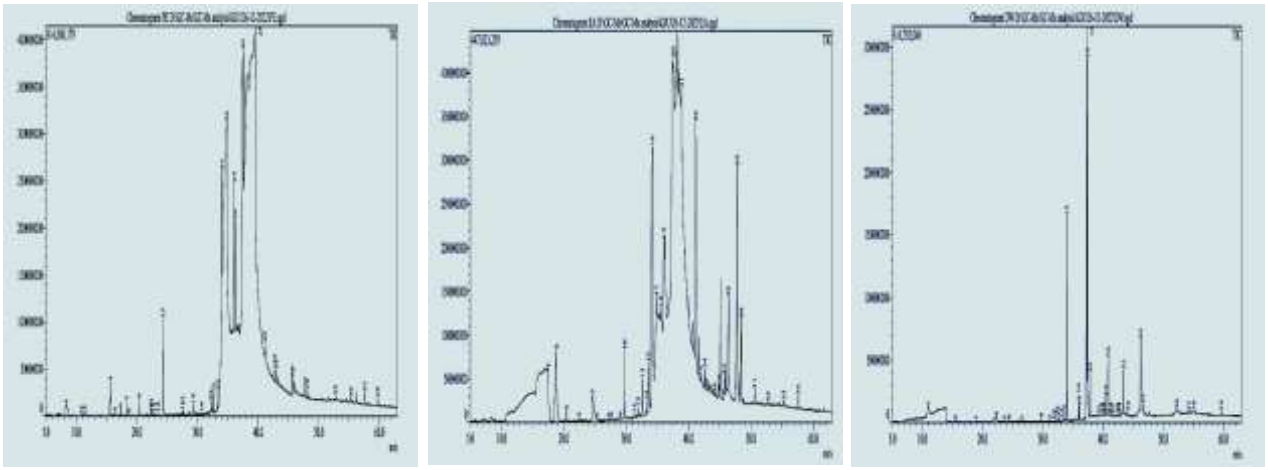
Ninteen phytochemicals were identified in the methanol: water (75:25) solvent mixture extract of nigella sativa as shown in table 14. Alpha-D-Galactopyranoside, 9-Octadecenoic acid and t-butylhydroquinone are the major phytochemicals of black seeds having the higher area percentage as 53.21%, 12.87% and 3.96%.

Figure 2 shows the chromatogram peaks of six single solvent (methanol, ethanol, acetone, petroleum ether, ethyl acetate and distilled water) extracts of nigella seeds. The compounds identified through mass spectrometry attached with GC with respect to their peak area and retention time. Total bioactive compounds were identified high in aqueous extracts, petroleum ether extracts, ethyl acetate extracts and ethanol extracts but major bioactive compounds with higher peak area were identified in methanol and acetone extracts. The major phytochemicals identified in methanol and acetone extracts were named as p-cymene-7-ol (49.64%), thymoquinone (14.70%), phenol (38.99%), linoleic acid (12.88%), t-butyl hydroquinone (8.29%), 2-Napthealenemethanol (2.14%), ethyl oleate (5.40%), 9-12-octdecadienoic acid, methyl ester (5.45%).

Among all the identified phytochemicals, linoleic acid, hexadecanoic acid is suggested to be fatty acid esters. Octdecadienoic acid is unsaturated fatty acid and has the antioxidant and anti-inflammatory property. The compound 9, 12- Octadecadienoic acid (Z,Z)-ethyl ester is a fatty acid ester and it may be employed as antioxidant, antimicrobial, flavor, hypo-cholesterolemic agent (Selvi *et al.*, 2016).



(A) Methanol Extract **(B) Ethanol Extract** **(C) Acetone Extract**

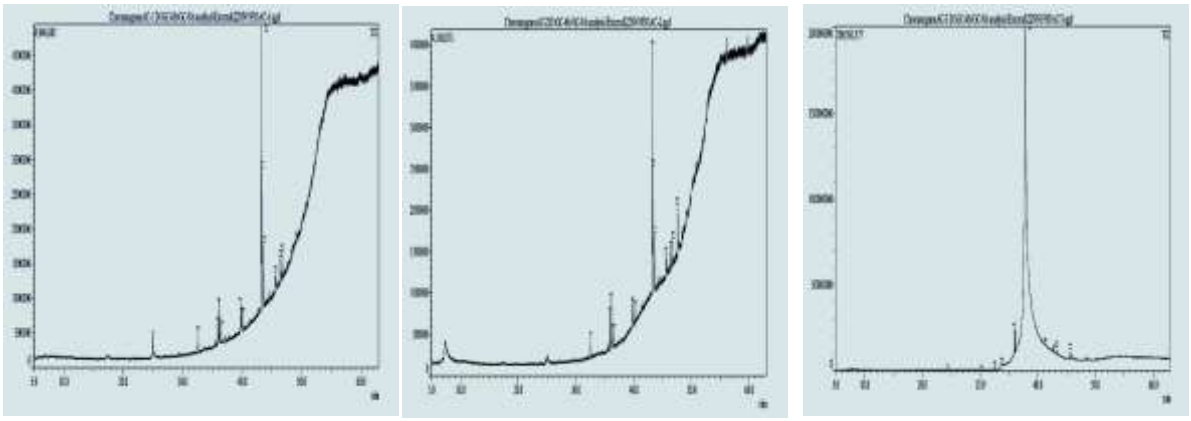


(D) Petroleum ether Extract **(E) Ethyl acetate Extract** **(F) Aqueous Extract**

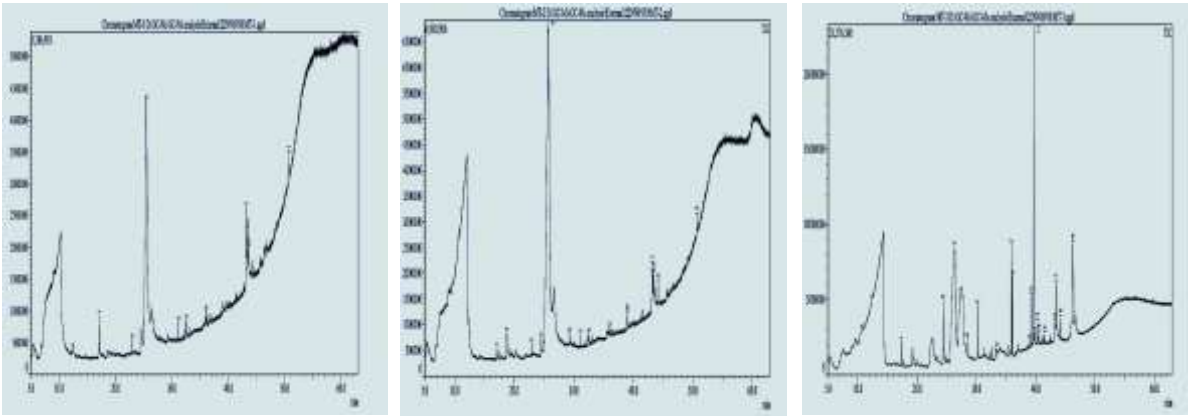
Figure: 2 Chromatogram showing peaks of different single solvent extracts of *Nigella sativa* seeds **(A)** Methanol Extract, (B) Ethanol Extract, **(C)** Acetone Extract, **(D)** Petroleum ether Extract, **(E)** Ethyl acetate Extract**, (F)** Aqueous Extract

Figure: 3 shows the chromatogram peaks of six extracts of two solvent mixtures (acetone and methanol with water) as acetone: water (25:75), acetone: water (50:50), acetone: water (75:25), methanol: water (25:75), methanol: water (50:50), methanol: water (75:25). The maximum number of 15 compounds was identified through GC-MS in solvent mixtures. As compared to single solvent extracts, solvent mixtures extracted a minimum number of phytochemicals. The phytochemicals were identified through GC-MS in solvent mixtures are given in tables 9, 10, 11, 12, 13 and 14. Compounds with higher peak area values are Alpha-

D-galactopyranoside (87.28%), thymol (6.22%), β-D-Ribopyranoside (2.52%), t-butyl hydroquinone (3.96%), Citronellal (2.42%). Among the identified phytochemicals, 13-Docosenoic acid, 9-Octadecenoic acid, 11-Eciosenoic acids are fatty acids. 15-Tetracosenoic acid is alcoholic compounds and it may be used as antimicrobial agent and hypocholesterolemic agents (Sarvana, 2013).



(A) Acetone Extract (25:75) **(B) Acetone Extract (50:50)** **(C) Acetone Extract (75:25)**



(D) Methanol Extract (25:75) **(E) Methanol Extract (50:50)** **(F) Methanol Extract (750:25)**

Figure: 3 Chromatogram showing peaks of different solvent mixtures' extracts of *Nigella sativa* seeds **(A)** Acetone: water Extract (25:75), (B) Acetone: water Extract (50:50), **(C)** Acetone: water Extract (75:25), **(D)** Methanol: water Extract(25:75), **(E)** Methanol: water Extract (50:50), **(F)** Methanol: water Extract(75:25).

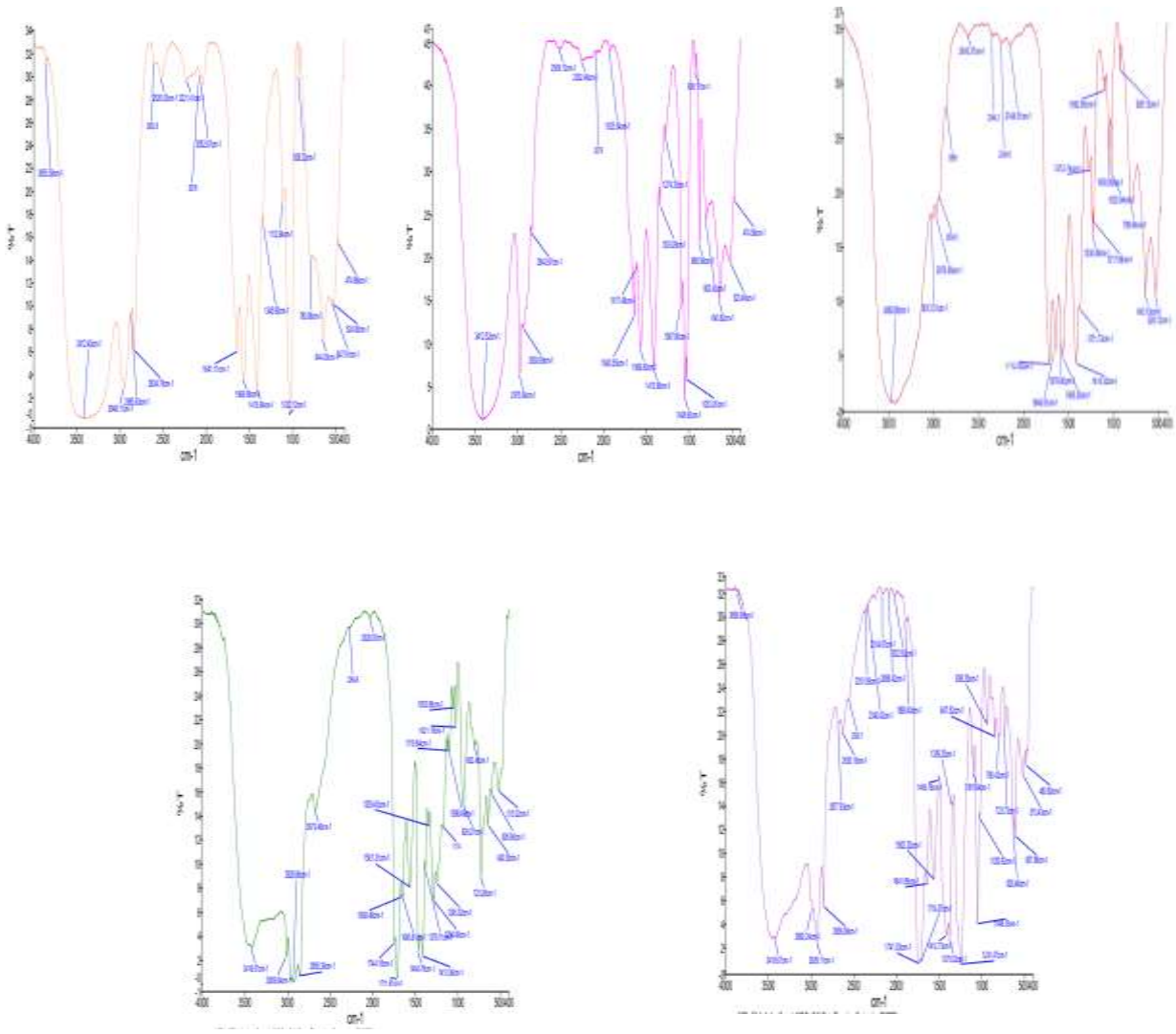


Figure: 4 FTIR spectra of black cumin seed extracts derived from various solvents displaying major functional groups of phyto-compounds. (a) methanol extract (b) ethanol extract (c) acetone extract (d) petroleum ether (e) ethyl acetate

In the present study, FT-IR analysis was performed to explore the major functional groups present in nigella seed extracts. Fig. 4 depicts the characteristic absorption bands in the range of 4000-500 cm^{-1}. The appearance of peaks at 3000-2800 cm^{-1}, represented stretching vibrations of the isopropyl and - CH_3 groups present in thymoquinone derivatives. More specifically, the peak at 2929.11cm^{-1} is attributable to C-H stretching of tertiary carbon in the isopropyl group, whereas peak at 2856.09 cm^{-1} belongs to the symmetric stretching modes of the three methyl groups. Rani et al. (2018) also confirmed the presence of characteristic FTIR absorption bands at 2924 cm^{-1} and 2854 cm^{-1} for thymoquinone.

6. Conclusion

Choosing an optimal solvent for efficient extraction of beneficial major bioactive compounds from black cumin depends on many factors from which the major one is the class of compounds need to be obtained. In this paper, we succeeded in determining optimal selection of solvents for efficient extraction of major bioactive compounds. Even though it was not easy to determine the optimal selection of solvents that would provide the higher concentration of major bioactive compounds. Methanol and acetone achieved by far the best results through the presence of higher number of major bioactive compounds in black cumin seed extracts. The presence of different phyto-constituents was determined through phytochemical screening in nigella sativa seed extracts. Higher numbers of phytochemicals (39 phytochemicals) were identified in seed extracts derived from single solvents while 20 phytochemicals were identified in solvent mixtures. That's why individual solvents are better choice for efficient extraction as compared to solvent mixtures. Also, GC-MS analysis results showed the higher presence of phytochemicals in methanol and acetone extracts and low phytochemicals were determined in extracts derived from combination of solvents.

In summary, the conducted study is highly promising towards the extraction mode as well as selection of solvents which affects the presence and extraction efficiency of phytochemicals. This study gives useful and valuable information for efficient extraction of major bioactive compounds and the end result of the study will help the researchers in optimal selection of solvents for efficient extraction of major phytochemicals from *Nigella sativa* seed extracts. As far as the authors are aware, this study is the first one for optimal selection of solvents from individual solvents and their combinations. The results indicated that major bioactive compounds of nigella could benefit many industries such as pharmaceutical, cosmetic and food industries along with the economy.

Credit authorship contribution statement

Nita Kaushik: Performed the experiments, Analyzed and interpreted the data, Literature search, Writing of the original research article

Aradhita Barmanray: Supervision, Investigation, Reviewed the paper and final approval.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

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