ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Short Communication

GAS CHROMATOGRAPHY - MASS SPECTROMETRY ANALYSIS OF THE ESSENTIAL OIL FROM *NIGELLA SATIVA* LINN. SEEDS

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ABSTRACT

Essential oil from *Nigella sativa* Linn. seeds was isolated and studied by Gas Chromatography-Mass Spectrometry (GC-MS). Yield of essential oils was 0.32%, while the rest 98.54 % were other phytochemicals. About 21 peaks corresponding to different compounds were identified in the *Nigella sativa* Linn. essential oil. Various compounds in the essential oil were identified by comparing their mass spectra fragmentation pattern with those of the NIST library and also with those of standard. The major components of the essential oil obtained were 13-octadecenoic acid, 9, 12 octadecadienoate (Z, Z), 2-Hydroxy-1-(hydroxymethyl) ethyl ester, 6-octadecenoic acid, and O-Cymene. Apart from that, alpha phellandrene, Thymoquinone, alpha-pinene, D-Limonene, Terpinene, Glycerol-1-palmitate, Longifoline, 4-methoxy thujane were also observed. Association of Official Analytical Chemists (AOAC) methods were followed in performing the proximate analysis of *Nigella sativa* Linn. seeds. In the proximate analysis, the contents of ash (3.34%), ethanol-soluble extractive (9.58%), water-soluble extractive (9.29%), loss on drying (4.66%) and, moisture (4.34%) were determined. Due to the presence of various phytochemical compounds, it is concluded that *Nigella sativa* Linn. seeds has a huge contribution in herbal drug manufacturing industries.

Keywords: *Nigella sativa* Linn., Essential oil, Gas Chromatography-Mass Spectrometry, Proximate analysis

1. INTRODUCTION

Spice is an edible part of the plant such as root, seed, bark, fruit, that enhances flavor, aroma, and tastes to food items [1]. Spices have a huge contribution not only in the food industry, but also in medicine or herbal drug formulation due to their number of medicinal properties, and a rich source of essential oil.

Nigella sativa Linn. (Family- Ranunculaceae); also called the black cumin is a miraculous herb with a religious background. For many decades, the plant is used as a medicine to cure various ailments showing a rich historical background. The plant is native to Southwest Asia, Africa, Europe and cultivated in Pakistan, Syria, Turkey, Saudi Arabia, India, and the Middle Eastern Mediterranean region [1, 2]. It is a dicotyledonous, annual, flowering plant that grows up to 90 cm tall. The fruit possesses several seeds that are black from outside and white inside. These seeds are 2 - 3. 5×1- 2 mm thickness, stimulant, diuretic, and used to treat puerperal fever. These seeds showed pharmacological activities such as analgesic, antispasmodic, and also CNS depressant activity [2]. In Unani and Ayurvedic medicines, these seeds play an important role to treat various diseases [3]. The seeds and its oil are shown to possess medicinal and pharmacological activities such as immunomodulatory [4], anticancer [5], antimicrobial [6], anti-inflammatory [7], gastroprotective [8], hepatoprotective [9], antioxidant [10], and renal protective properties [11]. *Nigella sativa* Linn. seeds are the best remedy to cure bronchial asthma [12]. It is not only useful in the treatment of nervous disorders, digestive problems such as diarrhea, but also cures dental problems [1]. The seeds are a boon for pregnant and lactating women as it increases milk production. From above properties, one can say that, the *Nigella sativa* Linn. seeds helps to maintain the overall immune system [1,13]. The essential oil of *Nigella sativa* Linn. seeds showed antioxidant activity [14] and antibacterial activity [15-19]. Across the world, in food preparations, *Nigella sativa* Linn. seeds are used as a spice and impart flavor to various food dishes due to its strong peppery taste and aroma [20]. As studied before, the seeds contain mainly fixed oils, alkaloids, saponins, and essential oil that was characterized by a higher percentage of monoterpenes. The chemical compound, namely, Thymoquinone, is the main constituent of the

essential oil of *Nigella sativa* Linn. seeds [21]. However, various factors such as the origin of the plant [22], and the type of extraction [23] are responsible for variations in the chemical composition of essential oil of *Nigella sativa* Linn. seed. The essential oils isolated from the plant part, are highly volatile, and hence, various methods of extraction such as hydrodistillation, steam distillation, and direct steam distillation are generally employed. Though GC-MS analysis of *Nigella sativa* Linn. is reported previously [24,25], the present work deals with the proximate analysis of the *Nigella sativa* Linn. seeds and isolation and analysis of chemical constituents in *Nigella sativa* Linn. using GC-MS analysis.

2. MATERIAL AND METHODS

2.1. Plant material

Nigella sativa Linn. seeds were collected from Krishi Kendra, Nagpur, India, was identified botanically and authenticated from the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India (Authentication number- 9990). The collected seeds were air-dried and then ground to a fine powder, sieved, weighed and, stored in an airtight container at room temperature for further analysis.

2.2. Proximate analysis

In proximate analysis, *Nigella sativa* Linn. seeds powder was examined for ash content, moisture content, loss on drying, extractive matter, according to the AOAC procedure [26].

2.3. Isolation of Essential Oil from *Nigella sativa* Linn. seeds

For the isolation of essential oil, about 100 g fine powder of *Nigella sativa* Linn. seeds were exhausted by hydrodistillation for 7-8 hours using Clevenger's apparatus. The oil obtained was further concentrated under reduced pressure. To avoid any phytochemical loss, the concentrated pale yellow colored essential oil obtained was stored in a dark colored bottle and kept in the refrigerator for further GC-MS analysis.

2.4. Chromatographic conditions

THERMO TRACE 1300 GC instrument was used for GC-MS analysis. The instrument was coupled with THERMO TSQ 8000 Mass Spectrometer and equipped with the column TG 5 MS having an internal diameter 30 m × 0. 25 mm and 0.25 µm film thickness were used. The column was composed of 5% Diphenyl and 95% dimethyl polysiloxane of non-polar nature and can tolerate 330-350°C. The carrier gas used was Helium (Purity-99.99%) with a flow rate of 1.0 ml/minute and with a split ratio of 10:1 mode was used for 1µl sample injector. The sample injector temperature was maintained at 250°C and the ion source temperature was set at 230°C. Also, the MS transfer line was maintained at 280°C. The oven temperature was programmed from 60°C for 2 minutes and then with an increase of 10°C to 280°C for 1.0 minutes. The ionization voltage of MS analysis was set at 70.0 eV with the scan interval of 0.5 seconds. Table 1 represents the chromatographic conditions that were set for GC-MS analysis of *Nigella sativa* Linn. essential oil.

The essential oil phytochemicals were determined by matching their mass spectra with those of standard and by matching their fragmentation pattern in MS with those of NIST library.The MS instrument determines the relative percentage area from the total area under the study.

Table 1: Chromatographic conditions for GC-MS analysis

GC-MS system	GC- THERMO TRACE 1300 MS- THERMO TSQ 8000
Detector	MS TSQ 8000
Stationary phase	TG 5MS capillary column
Carrier gas	Helium gas (99.99% purity)
Carrier flow	1 ml/min
Injection volume	1.0 µL
Column length	30 m X 0.25 mm
Particle size packing	0.25µm
S/SL	10:1
Injector temperature	250°C
MS transfer line temperature	280°C
Ion source temperature	230°C
Oven program	--- 60°C 2.0 min. 10°C 280°C 1.0 min.
Library used	NIST

3. RESULT

3.1. Proximate analysis

In the Proximate analysis, the ash content, percent ethanol-soluble extractives, percent water-soluble extractives, loss on drying, and moisture in the seeds of *Nigella sativa* Linn. was estimated as 3.34%, 9.58%, 9.29%, 4.66%, and 4.34% respectively. Table 2

represents the proximate analysis of *Nigella sativa* Linn. seeds.

Table 2: Proximate analysis of *Nigella sativa* Linn. Seeds

Parameters	Results
Ash value	3.34± 1.21 %
Percent Ethanol-soluble extractive	9.58 ± 0.30
Percent Water-soluble extractive	9.29 ± 1.46
Loss on drying	4.66 ± 1.23 %
Moisture	4.34 ±1.07 %

3.2. Percent Yield

The percent yield of the concentrated and pale yellow colored essential oil of *Nigella sativa* Linn. seeds obtained was 0.34.

3.3. GC-MS Analysis

To estimate various phytochemicals the GC MS analysis of concentrated form of essential oil of *Nigella sativa* Linn. seeds was carried out. Figure 1 represents the chromatogram obtained from GC-MS analysis of essential oil of *Nigella sativa* Linn. seeds.

As shown in the Table 3, a total 21 peaks indicating 21 different compounds were identified in the essential oil of *Nigella sativa*Linn. About 98.54 % of the essential oil of *Nigella sativa* Linn. was identified and estimated. The results of volatile contents obtained from the analysis were compared with the standards given by NIST and accordingly, the compounds were identified. The major components of essential oil of *Nigella sativa* Linn. seeds were 13-octadecenoic acid (32.98%), 9,12 octadecadienoate (Z, Z), 2-Hydroxy-1-(hydroxymethyl) ethyl ester (14.32%), O-cymene (13.47%), 6-octadecenoic acid (11.26%), Thymoquinone (6.42%), Alpha Phellandrene (5.61%), and Glycerol-1-palmitate (2.6%), that contributed about 80% of the essential oil. Apart from that, t- Butyl hydroquinone (1.44%), 13-Docosenamide (1.19%), Alpha pinene (1.18%), 3 Carene (1.15%), 4- methoxy thujane (1.09%), Trans-Geranylgeraniol (1.05%), and Longifoline (0.97%), were also observed in lower percentage as shown in Table 3.

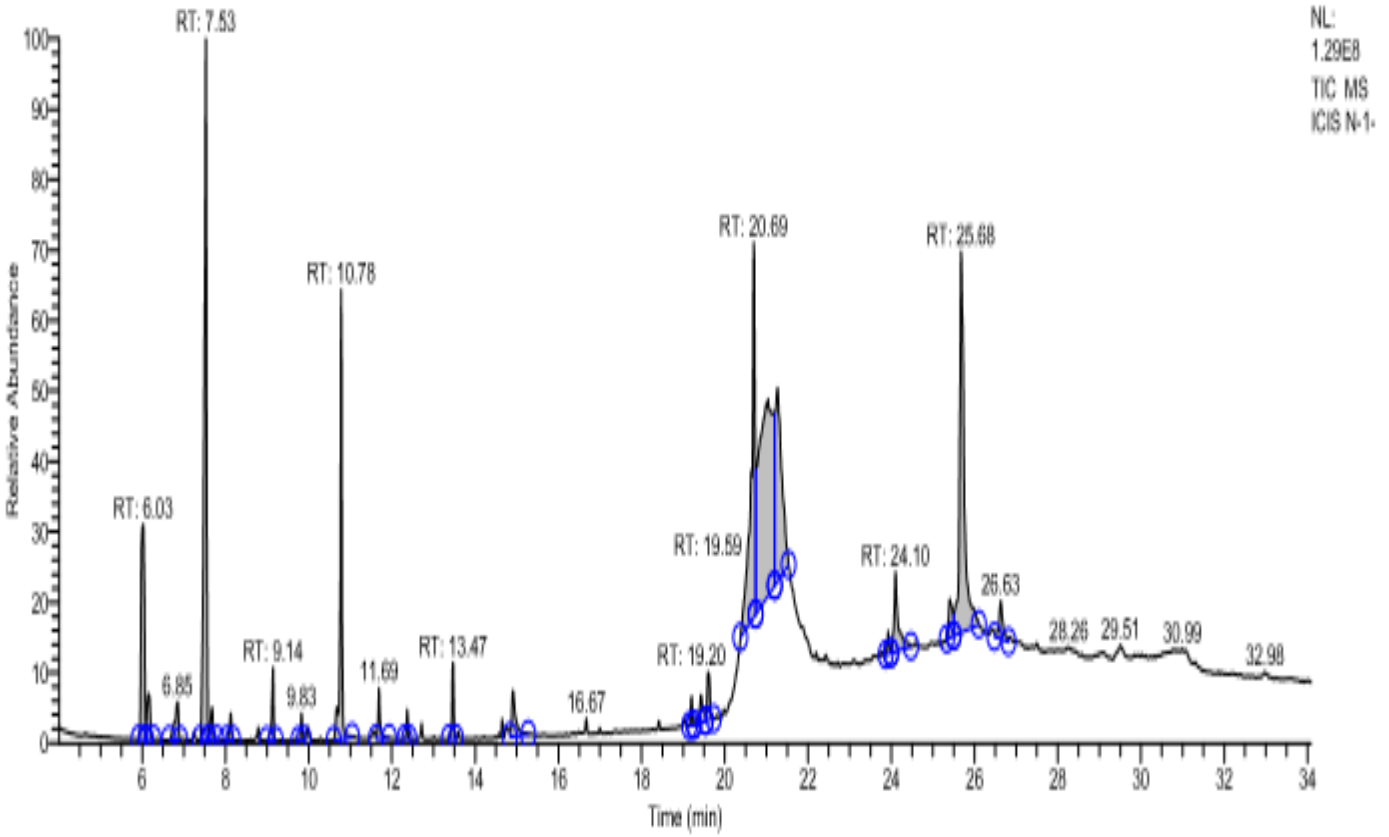

Fig. 1: GC-MS Chromatogram of *Nigella sativa* Linn. essential oil

Table 3: GC-MS analysis of essential oil of *Nigella sativa* Linn. Seeds

Sr.	RT	% Area	Name of the compound	Molecular Formula
1	6.03	5.61	Alpha Phellandrene	$C_{10}H_{16}$
2	6.16	1.15	3-Carene	$C_{10}H_{16}$
3	6.85	1.18	Alpha pinene	$C_{10}H_{16}$
4	7.53	13.47	O- Cymene	$C_{10}H_{14}$
5	7.67	0.65	D-Limonene	$C_{10}H_{16}$
6	8.13	0.44	Terpinene	$C_{10}H_{16}$
7	9.14	1.09	4- methoxy thujane	$C_{11}H_{20}O$
8	9.83	0.36	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$
9	10.78	6.42	Thymoquinone	$C_{10}H_{12}O_2$
10	11.69	0.83	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$
11	12.37	0.34	Cyclohexasiloxane dodecamethyl-	$C_{12}H_{36}O_6Si_6$
12	13.47	0.97	Longifoline	$C_{15}H_{24}$
13	14.91	1.44	t-Butyl hydroquinone	$C_{10}H_{14}O_2$
14	19.20	0.32	Phthalic acid, butyl hex-3-yl ester	$C_{18}H_{26}O_4$
15	19.43	0.87	Hexanoic acid,undec-10-enyl-ester	$C_{17}H_{32}O_2$
16	19.59	1.05	Trans-Geranylgeraniol	$C_{20}H_{34}O$
17	20.69	11.26	6-Octadecenoic acid	$C_{18}H_{34}O_2$
18	21.04	32.98	13-Octadecenoic acid	$C_{18}H_{34}O_2$
19	24.10	2.6	Glycerol-1-Palmitate	$C_{19}H_{38}O_4$
20	25.68	14.32	9,12-Octadecadienoic acid (Z, Z), 2-Hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{38}O_4$
21	26.63	1.19	13-Docosenamide	$C_{22}H_{43}NO$

4. DISCUSSION

In the proximate analysis, the moisture content determination in spices helps to determine the safe use of spices as, high moisture content makes the spice prone to attack by molds and bacteria thereby decreases the quality of the spices. The moisture content found was 4.34 ±1.07%, which is slightly less than the previously studied values [24, 27]. Also, ash values studied previously [27, 28] were found approximately equal to studied in this experiment as 3.34 ± 1.21%. From Table 3, the chemical compounds are identified in *Nigella sativa* Linn. essential oil contains major percentages of the fatty acid compound, namely 13-Octadecenoic acid and 6-Octadecenoic acid. It also contains a high percentage of 9, 12 octadecadienoate (Z, Z), 2-Hydroxy-1-(hydroxymethyl) ethyl ester. As reported earlier, Thymoquinone is one of the major constituent found in the *Nigella sativa* Linn. seeds that show anticarcinogenic, anti-inflammatory, and antioxidant activity [21]. In our study, the percent content of Thymoquinone was found as 6.42. Apart from that, the essential oil is the source of Terpenes such as alpha phellandrene, alpha-pinene, D-Limonene, and also Quinones such as t-Butyl hydroquinone but, the occurrence of Terpenes, Quinones and Thymoquinone are slightly less than the previously studied values [24]. These phenolic and terpene compounds obtained in this essential oil kill various microorganisms hence, acts as antimicrobial agent [29,30]. The variations in chemical compounds found in the essential oil depend on the type of soil in which the plant grows climatic conditions, and fertilizers. The volatile contents found in the essential oil of *Nigella sativa* Linn. contribute to various medicinal uses, thereby, used in herbal drug manufacturing. From the proximate analysis values of the *Nigella sativa* Linn. seeds shows that the seeds are safe for human consumption. From the GC-MS analysis, it is clear that essential oil obtained from *Nigella sativa* Linn. seeds are a rich source of phytochemicals that shows a huge application in herbal formulations as it shows various activities such as, anti-inflammatory, antibacterial, antidiabetic, anticancer, and analgesic.

5. ACKNOWLEDGEMENTS

The author (P. G. Bhishnurkar) thankful to the Director, Institute of Science, Nagpur for providing the necessary facilities to carry out the research work. The

authors are also thankful to CIL/ SAIF, Punjab University, Chandigarh for carrying out GC-MS analysis.

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