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RESEARCH ARTICLE

GC-MS ANALYSIS OF FIXED OILS OF *NIGELLA SATIVA* SEEDS

Sania Feroz*, Ghias Uddin
Institute of Chemical Sciences, University of Peshawar, K.P.K Peshawar-25120, Pakistan.

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***Address for Correspondence:**
Sania Feroz, Institute of Chemical Sciences, University of Peshawar, K.P.K Peshawar-25120, Pakistan, E-mail: *muskroz1@gmail.com*

Abstract

Objective: Gas chromatography-Mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples.
Methods: The present study also relies on use of GC-MS for detection and interpretation of compounds present in *N. sativa* oil samples. Fixed oil was obtained through column chromatography of ethyl acetate fraction. The oil samples were subjected to GC-MS analysis which showed 5, 18, 12 and 20 compounds in four fixed oil samples respectively.
Results: The major components were linoleic acid, methyl ester (35.5%), oleic acid, methyl ester (15.007%), palmitic acid, methyl ester (8.208%).
Conclusion: Study concludes that in fixed oils, linoleic acid constitutes the major portion while oleic acid and palmitic acid also contributes in small quantity.
Keywords: Column chromatography, fixed oil, GC-MS, *Nigella sativa*.

INTRODUCTION

Plant-derived substances are now being widely used as medicines as these have recently become of great interest owing to their versatile applications. Medicinal plants are the richest natural bio-resource of drugs of traditional systems of medicine. With the advancement in research medicinal plants are considered a source of modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[1]. Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. Oilseeds are important sources of oils of nutritional, industrial and pharmaceutical importance. Non-conventional oilseeds are being considered because their constituents have unique chemical properties and may augment the supply of edible oils[2]. The study of oilseeds for their minor constituents is useful in order to use both oil and minor constituents effectively.
Nigella sativa, which belongs to the family Ranunculaceae, commonly grows in Eastern Europe, the Middle East, and Western Asia[3]. It is a small shrub with tapering green leaves and bearing white and purplish flowers. Its ripe fruit contains tiny black seeds, commonly known as black seeds in English[4]. Seeds of *N. sativa* are frequently used in folk medicine in the Middle East and some Asian countries for the promotion of good health. Seeds are used for the treatment of various diseases including fever, the common cold, headache, asthma, rheumatic diseases, and microbial infections and to expel worms from the intestines as well as cancer. In addition, it is used as a flavoring additive to bread and pickles[5]. The seeds contain a yellowish volatile oil, a fixed oil, proteins, amino acids, reducing sugars, mucilage, alkaloids, organic acids, tannins, resins, toxic glycoside, glycosidal saponins, crude fiber, minerals, and vitamins[6]. The aim of the present study was to find the composition of fixed and volatile oils obtained from ethyl acetate fraction.

MATERIALS AND METHODS

Plant Material: The seeds of *N. sativa* were purchased from a local spice market of Peshawar, KPK Pakistan.
Plant Identification: The purchase seeds of *N. sativa* were identified by a botanist, Prof. Dr. Abdur Rashid, in Department of Botany, University of Peshawar, KPK Pakistan.
Extraction and fractionation: The seeds were grinded in a rotary mill and crude extract was obtained. This extract was fractionated with polar and non polar solvents which were methanol, ethyl acetate, chloroform and *n*-hexane respectively. Then, each

fractionated sample was concentrated in rotary evaporator and solvent was removed to obtain concentrated extract[7].

GC–MS analysis of fixed oil: A Shimadzu gas chromatograph, hyphenated to a QP2010 plus (Tokyo, Japan) mass spectrometer, outfitted with an auto-injector (AOC-20i) and auto sampler (AOC-20S) was used. The carrier gas used was Helium and a capillary column TRB-FFAP of specification: length; 30m, thickness; 0.250 µm, i.d.; 0.35 mm and treated with polyethylene glycol was used for all chromatographic separations. Other GC-MS parameters are: pressure: 100KPa, temperature: 240ºC, solvent cut time; 1.6 min. 1µl of standard and sample were injected into the column of GC. The injector operatory mode was a split mode, with a split ratio of 1:50, and 240ºC as an injection temperature. Initially the column temperature was 50ºC and was changed at the rate of 15ºC for each minute and raised to 150ºC. After 150ºC, the rising rate of temperature was 2.5ºC per minute and was raised to 175ºC and was maintained for 5 minutes. Then, the rising rate of temperature was 2.5ºC per minute at which the temperature was to 220ºC[8].

MS scanning was executed from m/z 85 to m/z 380. GC-MS solutions software, provided by the supplier was used for the system control and acquiring the data. Compounds identification was carried out by the comparison of the relative retention times of the components and obtained mass spectra with standard mass spectra (from the NIST library, NIST 05).

RESULTS AND DISCUSSION

Four samples of fixed oils were obtained and were subjected to GC-MS analysis. GC-MS analysis confirmed the presence of various compounds in fixed oils in different ratio. GC-MS analysis of fixed oils is illustrated in Figure 1, 2, 3, and 4 respectively. The graphs are also illustrated in tabular forms in Table 1, Table 2, Table 3 and Table 4 respectively. Current study has reported the chemical composition of fixed oils of *N. sativa*. GC-MS analysis of oils confirmed the presence of various compounds in them. Four samples of fixed oils were obtained and subjected to GC-MS analysis. In the first sample, all methyl esters were present in very small quantity (Table 1).

Table 1: GC-MS analysis of *N. sativa* fixed oil (sample 1).

S. N.	Name	R. Time	Area	Con. (%)
1.	C12:0; Lauric acid, methyl ester	8.085	4794	0.005
2.	C14:0, Myristic acid, methyl ester	10.175	7114	0.007
3.	C16:0, Palmitic acid, methyl ester	13.410	43880	0.042
4.	C18:0, Stearic acid, methyl ester	17.851	25010	0.024
5.	C18:1c, Oleic acid, methyl ester	18.262	8149	0.028

Table 2: GC-MS analysis of *N. sativa* fixed oil (sample 2).

S. N.	Name	R. Time	Area	Conc. (%)
1.	C6:0; Hexanoic acid, methyl ester	2.944	19619	0.058
2.	C8:0; Caprylic acid, methyl ester	4.743	3433	0.006
3.	C10:0; Capric acid, methyl ester	6.503	12385	0.017
4.	C11:0; Undecanoic acid, methyl ester	7.290	1456	0.002
5.	C12:0; Lauric acid, methyl ester	8.084	21023	0.026
6.	C13:0; Tridecanoic acid, methyl ester	9.010	2152	0.003
7.	C14:0; Myristic acid, methyl ester	10.176	116414	0.136
8.	C15:0; Pentadecanoic acid, methyl ester	11.636	15267	0.018
9.	C16:0; Palmitic acid, methyl ester	13.420	2086913	2.380
10.	C17:0; Margaric acid, methyl ester	15.499	15200	0.018
11.	C18:0; Stearic acid, methyl ester	17.859	517160	0.599
12.	C18:1c; Oleic acid, methyl ester	18.288	900188	3.764
13.	C18:1n9t; Elaidic acid, methyl ester	18.471	35271	0.202
14.	C18:2c; Linoleic acid, methyl ester	19.572	2739870	10.092
15.	C18:2t; Octadecadienoic acid, methyl ester	19.733	15718	0.056
16.	C18:3n3; Linolenic acid, methyl ester	21.669	11169	0.044
17.	C20:0; Arachidic acid, methyl ester	24.618	20659	0.025
18.	C20:2c; 11,14-Eicosadienoic acid, methyl ester	26.942	90808	0.319

Table 3: GC-MS analysis of *N. sativa* fixed oil (sample 3).

S. N.	Name	R. Time	Area	Conc. (%)
1.	C6:0; Hexanoic acid, methyl ester	2.945	2382	0.006
2.	C12:0; Lauric acid, methyl ester	8.084	5334	0.006
3.	C14:0; Myristic acid, methyl ester	10.176	20552	0.020
4.	C15:0; Pentadecanoic acid, methyl ester	11.637	6708	0.007
5.	C15:1; Pentadecenoic acid, methyl ester	12.012	5410	0.025
6.	C16:0; Palmitic acid, methyl ester	13.413	201325	0.195
7.	C16:1; Palmitoleic acid, methyl ester	13.883	9593	0.046
8.	C17:0; Margaric acid, methyl ester	15.504	6276	0.006
9.	C18:0; Stearic acid, methyl ester	17.853	60741	0.060
10.	C18:1c; Oleic acid, methyl ester	18.268	59189	0.211
11.	C18:1n9t; Elaidic acid, methyl ester	18.469	6962	0.034
12.	C18:2c; Linoleic acid, methyl ester	19.533	70260	0.220

GC-MS analysis of second sample showed that linoleic acid was the major component which was 10% followed by oleic acid and palmitic acid which are 3.76% and 2.38% respectively while other compounds were in small quantity (Table 2). Analysis of third sample showed that various methyl esters were present but in small amounts (Table 3). GC-MS analysis of fourth sample explored that linoleic acid (35.55%) was the major component while oleic acid (15.007%), palmitic acid (8.20%) and stearic acid (1.877%) were also present. Many other components were present in minute quantities (Table 4).

Table 4: GC-MS analysis of *N. sativa* fixed oil (sample 4).

S. N.	Name	R. Time	Area	Conc. (%)
1.	C6:0; Hexanoic acid, methyl ester	2.945	12976	0.039
2.	C8:0; Caprylic acid, methyl ester	40744	1355	0.002
3.	C12:0; Lauric acid, methyl ester	8.085	5679	0.007
4.	C13:0; Tridecanoic acid, methyl ester	9.187	1808	0.002
5.	C14:0; Myristic acid, methyl ester	10.180	109863	0.128
6.	C15:0; Pentadecanoic acid, methyl ester	11.641	20937	0.025
7.	C15:1; Pentadecenoic acid, methyl ester	12.017	11790	0.065
8.	C16:0; Palmitic acid, methyl ester	13.448	7199712	8.208
9.	C16:1; Palmitoleic acid, methyl ester	13.895	29472	0.165
10.	C17:0; Margaric acid, methyl ester	15.513	31426	0.038
11.	C17:1; Heptadecenoic acid, methyl ester	15.956	2716	0.015
12.	C18:0; Stearic acid, methyl ester	17.897	1621340	1.877
13.	C18:1c; Oleic acid, methyl ester	18.354	3590155	15.007
14.	C18:1n9t; Elaidic acid, methyl ester	18.506	172499	0.987
15.	C18:2c; Linoleic acid, methyl ester	19.680	9657037	35.555
16.	C18:2t; Octadecadienoic acid, methyl ester	19.742	99458	0.353
17.	C18:3n3; Linolenic acid, methyl ester	21.689	32240	0.126
18.	C20:0; arachidic acid, methyl ester	24.637	70834	0.086
19.	C20:1c; 11-Eicosenoic acid, methyl ester	25.215	55869	0.245
20.	C20:2c; 11,14-Eicosadienoic acid, methyl ester	26.970	432857	1.518

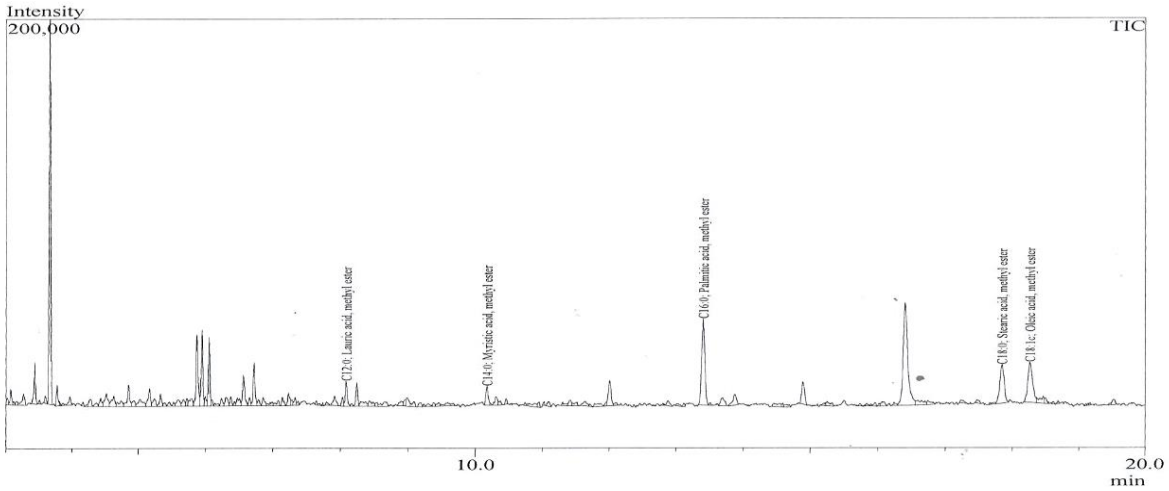

Figure 1: Graphical representation of GC-MS analysis of sample 1.

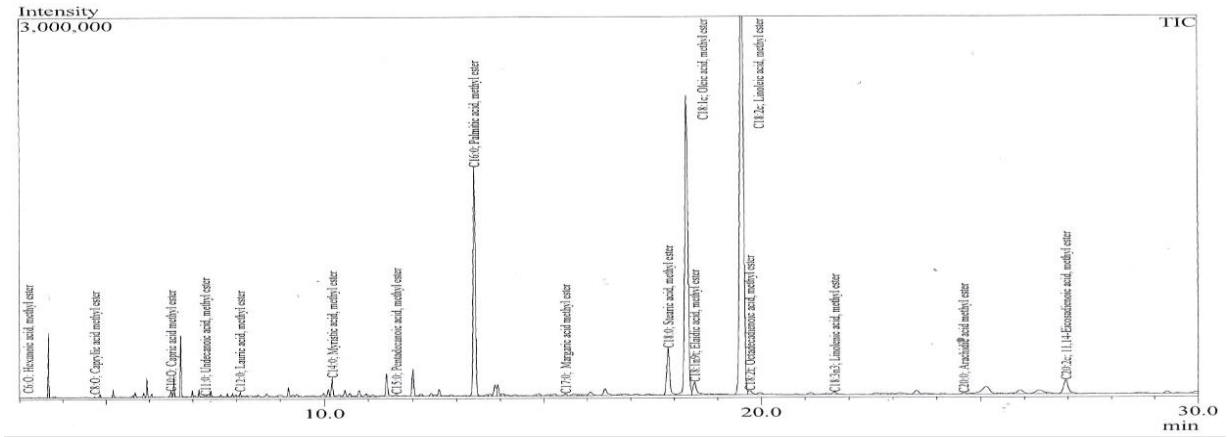

Figure 2: Graphical representation of GC-MS analysis of sample.

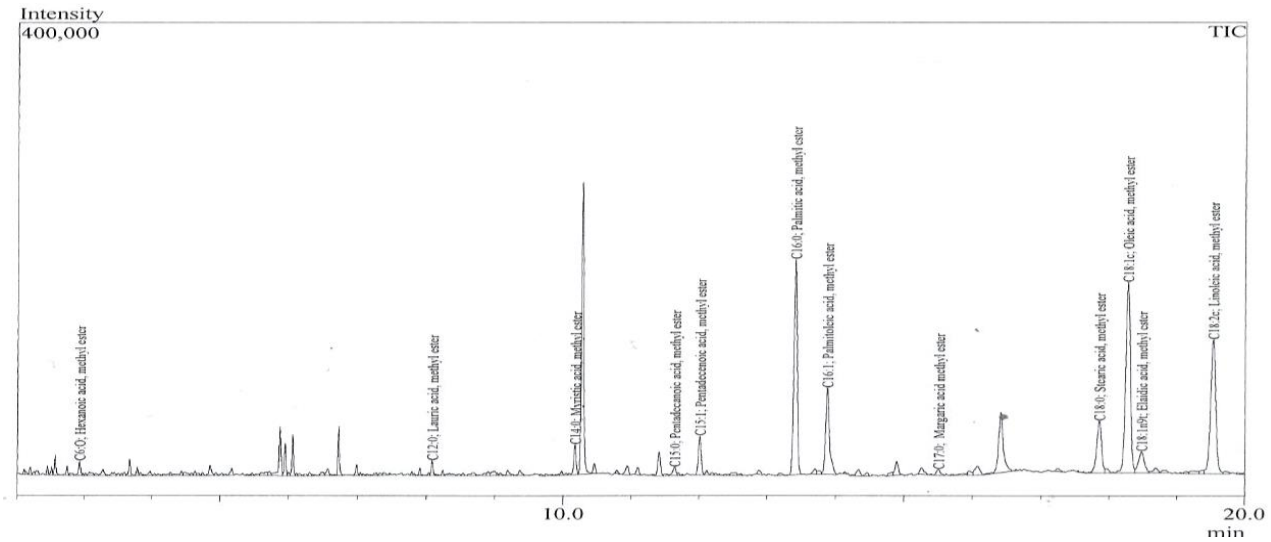

Figure 3: Graphical representation of GC-MS analysis of sample 3.

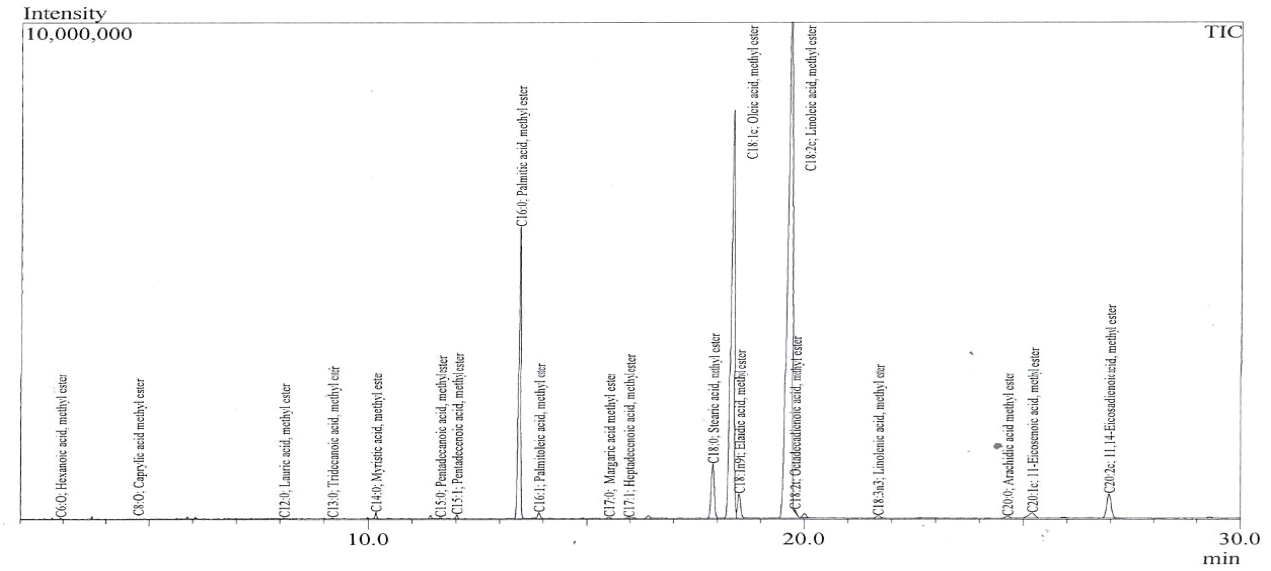

Figure 4: Graphical representation of GC-MS analysis of sample 4.

CONCLUSION

Study concludes that in fixed oils, linoleic acid constitutes the major portion while oleic acid and palmitic acid also contributes in small quantity. Many other components are also present in very minute amount.

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AUTHOR'S CONTRIBUTION

Feroz S: writing original draft, methodology, investigation. **Uddin G:** formal analysis, data curation, conceptualization. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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