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. Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. Oils 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. 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. 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. 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. 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. 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.

**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.; 0.35mm and treated with polyethylene glycol was used for all chromatographic separations. Other GC–MS parameters are: pressure: 100KPa, temperature: 240°C, 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 240°C as an injection temperature. Initially the column temperature was 50°C and was changed at the rate of 15°C for each minute and raised to 150°C. After 150°C, the rising rate of temperature was 2.5°C per minute and was raised to 175°C and was maintained for 5 minutes. Then, the rising rate of temperature was 2.5°C per minute at which the temperature was to 220°C.

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).

| Table 1: GC–MS analysis of *N. sativa* fixed oil (sample 1) |
|-----------------------------------------------|
| S. N. | Name | R. Time | Area | Conc. (%) |
|------|------|---------|------|-----------|
| 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; Arachidic acid, methyl ester | 19.572  | 2739870 | 10.092 |
| 15.  | C18:2t; Octadecadienoic acid, methyl ester | 19.733  | 15715 | 0.086 |
| 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     |

**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 have 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). 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).

**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.

### 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  | 9657037| 35.555    |
| 15.   | C18:2n6t; Linoleic acid, methyl ester     | 19.742  | 99458  | 0.353     |
| 16.   | C18:3n3; Linolenic acid, methyl ester     | 21.689  | 32240  | 0.126     |
| 17.   | C20:0; arachidic acid, methyl ester       | 24.637  | 70834  | 0.086     |
| 18.   | C20:1c; 11-Eicosenoic acid, methyl ester  | 25.215  | 55869  | 0.245     |
| 19.   | 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
AUTHOR’S CONTRIBUTION
The manuscript was carried out, written, and approved in collaboration with all authors.

CONFLICT OF INTEREST
No conflict of interest associated with this work.

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