Chemical Composition of Essential Oils of Released Black Cumin Varieties Grown in Ethiopia

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Abstract
In Ethiopia black cumin seed and oil are used in folk medicine, as a bread flavoring and as a spices. The essential oil of black cumin seeds were extracted by hydro-distillation with Clevenger apparatus and the constituents of the three varieties were identified using GC/MS. The essential oil content of black cumin seeds were 0.80%, 0.4% and 0.6% for Eden, Dershy and Silengo respectively. The GC/MS results showed that seventy five, sixty six and sixty constituents were identified which constituting 99.98%, 99.27% and 99.17% in Eden, Dershy and Silengo. The main constituents of Ethiopian black cumin verities  seeds essential oil as detected by GC/MS ware p-cymene (45.85 - 44.31%), α-thujen (17.30 - 12.57%), trans-4-methoxy thujane (8.86 - 7.39%), 9,12-Octadecanoic acid, (Z,Z)- (6.04% - 0.07%), β-pinene (4.08 - 3.04%), α- pinene (3.94 - 2.68%), gamma-terpinene (3.83 - 2.50%), thymoquinone (3.53 - 2.13%), α-terpinene (3.00 – 0.00%), D-limonene (2.29 - 2.08%), phenol-2-methyl-5-(1-methylethyl) (2.56 -1.52%), cis-vaccenic acid (2.46 - 0.00%), longifolene (1.95 - 1.83%), the results confirms that main compounds are similar and variation in content to each varieties. The Ethiopian black cumin essential oil contain the required major secondary metabolite for pharmacological and other application.

Keywords: Black cumin, Ethiopia, Essential oil, p-cymene,spices

1. Introduction
A Spice is a natural compound that is extracted from the seed, fruits, flowers or trunks of several plants are add to food in order to provide taste smell or flavor. Spices are a diverse group of wide variety of staple dietary additives consumed all over the world. The spice is a culinary term not a botanical category it does not refer to a specific kind of plant or plant part [1].

Each spice has a unique aroma and flavor which derive from compounds known as phytochemical or secondary metabolite. These chemicals evolved in plant to protect them against herbivorous insect, vertebrates, fungi pathogens and parasites [2-4].

Black cumin or Nigella sativa L. (N. sativa) belongs to the family Ranunculaceae and is widely distributed and cultivated in Mediterranean countries, middle Europe and western Asia. The ancient Egyptians, Greeks and Romans were already aware of the therapeutic properties of N. sativa, the essential oil and seeds of which are still used in folk medicine, as a bread or cheese flavoring and as a spice in various kinds of meals [5].

The seeds have been reported to contain mainly fixed oils, proteins, alkaloids, saponins, and essential oil that was previously characterized by a higher percentage of monoterpenes, the main constituents being thymoquinone and p-cymene [6].

Black seed, the seed of Nigella sativa L. (Ranunculaceae), has been employed for thousands of years as a spice, food preservative and curative remedy for numerous disorders [7]. The historical tradition of black seed use in medicine is substantial. N. sativa is known to have beneficial effects on a wide range of diseases, antiasthmatic, antitumor, antiviral, antibacterial, anti-inflammatory, antimalarial, antihypertensive, antidiabetic, [8-12].

Thymoquinone, the main constituent of the essential oil of N. sativa seeds, was capable to also exert beneficial effects on acute gastric ulcer [13]. In addition, thymoquinone and its reduced product thymohydroquinone have been reported to have an antibiotic activity and beneficial interaction with some antibiotics [14]. In Ethiopia black cumin seed and oil are used in folk medicine, as a bread flavoring and as a spices. The aim of this study is to describe the chemical composition of essential oil black cumin seeds cultivated in Ethiopia.

2. MATERIALS AND METHODS
2.1. Isolation of essential oils ;
The collected seeds were air dried, ground with a laboratory mill, weighed and particular samples (100g) from each varieties were subsequently submitted to hydro-distillation for 4h using Clevenger type apparatus. Three replicates were distilled simultaneously for each. All oil samples were weighed, dried over anhydrous sodium sulphat and stored in dark at 4°C to identification of chemical constituent. The yields obtained were averaged and calculated as a relative percentage (V/W).

2.2. Gas chromatography (GC)
GC analyses were performed by using Agilent 7890B gas chromatography equipped with a DB5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25µm). Oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injection and detector (FID) temperature were 260°C; Helium was used as carrier gas.

2.3. Gas chromatography- Mass spectrometry (GC-MS)
GC-MS analyses were carried out Agilent 5977A system equipped with a DB-5MS fused silica column (30 m x 0.25 mm; film thickness 0.25µm i.d.). Oven temperature was 40 to 240°C at a rate of 4°C/min, transfer line temperature 260°C, injector temperature 250°C, carrier gas Helium was used split ratio 1/60, flow rate 1 ml/min.

2.4. Identification of compounds
The constituent of the oils were identified by comparison of their mass-spectra with those of a computer NIST 2014. library and confirmed by comparison of their compounds. Adams, (1995). [15]. Retention indices were determined using retention times of n-al-kanes that have been injected to the same instrument and under the same chromatographic condition. Relative percentage amounts were calculated from the total area under the peak by the software of the apparatus.

3. Results and Discussions
Hydro-distillation of Ethiopian black cumin seeds were yellowish oil and resulted in 0.80%, 0.60% and 0.40% essential oil for Eden, Dershay and Silengo varieties respectively. The obtained result in this study was confirms that the variation in essential oil content with in variety and comparable with many authors report. Prabudha and Ria, [16] reported that the percentage of volatile oils of cumin seeds were ranged from 0.5 to 1.5%. Kokoska L. et al., (2008) [18] reported that the cumin seeds essential oil was 0.29%.

Table 1; Chemical composition of black cumin seeds varieties Essential oil

| No | Identified compounds               | Eden (%) | Dershy (%) | Silengo (%) |
|----|------------------------------------|----------|------------|-------------|
| 1  | P- Cymene                          | 44.36    | 45.85      | 44.31       |
| 2  | α-Thujane                          | 12.57    | 17.30      | 13.66       |
| 3  | trans-4-methoxy thujane           | 7.39     | 7.91       | 8.86        |
| 4  | 9,12-Octadecanoic acid, (Z,Z)-     | 6.04     | 0.07       | 0.16        |
| 5  | β-pinene                           | 3.04     | 4.08       | 3.45        |
| 6  | α-pinene                           | 2.68     | 3.94       | 3.00        |
| 7  | gamma-terpinene                    | 2.50     | 3.83       | 3.24        |
| 8  | cis-vaccenic acid                  | 2.46     | -          | -           |
| 9  | Thymoquinone                       | 2.13     | 2.40       | 3.53        |
| 10 | Longifolene                        | 1.83     | 1.56       | 1.95        |
| 11 | D-limonene                         | 2.08     | 2.29       | 2.27        |
| 12 | Phenol-2-methyl-5-(1-methylethyl)- | 1.60     | 1.18       | 2.56        |
| 13 | Bicyclo[3,1,0]hexane,4-methylethene-1-(1-methylethyl) | 1.52     | 2.29       | 1.87        |
| 14 | Terpinen-4-ol                      | 1.13     | 0.92       | 1.31        |
| 15 | cis-4-methoxy thujane             | 1.06     | 1.30       | 1.35        |
| 16 | n-hexadecanoic acid               | 0.96     | 0.02       | 0.04        |
| 17 | 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl) | 0.93 | - | - |
| 18 | 1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl | 0.91 | 0.93 | 1.00 |
| 19 | Behenic alcohol                    | 0.88     | 0.13       | 0.40        |
| 20 | 9,12-octadecadieenoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl)ethylster | 0.61 | 0.03 | 0.02 |
| 21 | 2-carene-4-ol                     | 0.34     | 0.03       | 0.43        |
| 22 | Tricyclo[5,4,0,0(2,8)undec-9-ene,2,6,6,9- tetramethylen-1(1R,2S,7R,8R) | 0.30 | 0.3 | 0.28 |
| 23 | Glycidyl olate                     | 0.22     | 0.02       | -           |
| 24 | Bicycle[2,2,1]heptan-2-ol,1,7,7-trimethyl-,acetate,endo  | 0.18 | 0.16 | 0.26 |
| 25 | p-Mentha-1,5,8-triene              | 0.15     | -          | 0.10        |
| 26 | Oleic acid                         | 0.13     | 0.03       | 0.06        |
| 27 | Isoledene                          | 0.12     | 0.1        | 0.12        |
| 28 | 7-Tetradecenal, (Z)               | 0.12     | 0.08       | 0.13        |
| 29 | Glycidyl palmitate                 | 0.10     | -          | -           |
| No | Identified compounds                                                                 | Eden (%) | Dershy (%) | Selingo (%) |
|----|----------------------------------------------------------------------------------------|----------|------------|-------------|
| 30 | Cyclohexanol, 1-methyl-4-(1-methylethenyl)-,cis-                                        | 0.10     | 0.10       |             |
| 31 | Benzenemethanol,a,a,4-trimethyl                                                         | 0.10     | 0.04       | 0.06        |
| 32 | (-)-Carvone                                                                             | 0.09     | 0.08       | 0.15        |
| 33 | Bicyclo[3,1,0]hexan-2-ol,2-methyl-5-(1-methyethyl)-(1α, 2β,5α)                         | 0.08     | 0.07       | 0.08        |
| 34 | Cyclohexene, 1-methyl-4-(methylthyl)                                                   | 0.08     | 0.09       | 0.08        |
| 35 | Eucalyptol                                                                             | 0.07     | 0.08       | 0.07        |
| 36 | 1,2,4-methenozulene,decahydro-1,5,5,8a-tetramethyl-[1S-(1α,2α,3αβ,4α,8αβ,9R*)]           | 0.07     | 0.06       | 0.08        |
| 37 | Caryophylyl                                                                            | 0.07     | 0.06       | 0.08        |
| 38 | Humulane-1,6-dien-3-ol                                                                  | 0.07     | 0.04       | 0.07        |
| 39 | p-Cymenene                                                                             | 0.06     | 0.06       | 0.08        |
| 40 | 1,2,15,16-diepoxyhexadecane                                                             | 0.06     |            |             |
| 41 | (1R,3R,4R,5S)-1-Isopropyl-4-methylbicyclo[3,1,0]hexan-3-yl acetal-rel-                  | 0.05     | 0.04       | 0.06        |
| 42 | Tetradecanol                                                                           | 0.06     | 0.04       | 0.08        |
| 43 | α-Terpineol                                                                            | 0.04     | 0.02       | 0.04        |
| 44 | Camphene                                                                               | 0.04     | 0.06       | 0.05        |
| 45 | α-phellandrene                                                                         | 0.04     | 0.06       | 0.06        |
| 46 | 2-Cyclohexen-1-ol,1-methyl-4-(methylthyl)-,cis                                         | 0.04     | 0.02       | 0.04        |
| 47 | 1H-Indene, 1,ethylideneoctahydro-7a-methyl-, cis-                                     | 0.04     | 0.04       | 0.07        |
| 48 | Benzene, 1-(1,5-dimethyl-4-hexenyl)4-methyl                                            | 0.04     | 0.04       | 0.06        |
| 49 | Ar-tumerone                                                                            | 0.04     | 0.04       | 0.10        |
| 50 | Phenanthrene, 7-ethyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl-[4aS-(4aα,4bβ,1β,10aβ)] | 0.04     | 0.02       | 0.04        |
| 51 | Tetradecanoic acid, 2-hydroxy                                                         | 0.04     | -          | -           |
| 52 | Bicycle[3,1,0]hex-2-ene,4-methylene-1-(1-methylethyl)                                  | 0.02     | 0.03       | 0.03        |
| 53 | β-ncyrcone                                                                             | 0.03     | 0.04       | 0.04        |
| 54 | Thymol                                                                                 | 0.03     | 0.02       | 0.04        |
| 55 | Cis-9-tetradecen-1-ol                                                                   | 0.03     | 0.02       | 0.07        |
| 56 | 2-Caren-4-ol                                                                           | 0.02     | 0.02       |             |
| 57 | 1,3,8-p-Menthatriene                                                                   | 0.02     | 0.02       | 0.02        |
| 58 | (+)-(E)-Limonene oxide                                                                  | 0.02     | 0.02       | 0.03        |
| 59 | β-Bisabolene                                                                           | 0.02     | 0.02       | 0.03        |
| 60 | trans-2-Decen-1-ol,trifluoroacetate                                                    | 0.02     | -          | -           |
| 61 | Dodecanal                                                                              | 0.02     | 0.01       | 0.04        |
| 62 | p-Cymene-2,5-diol                                                                       | 0.02     | 0.04       | 0.06        |
| 63 | Tumerone                                                                               | 0.02     | 0.02       | 0.04        |
| 64 | Cirlone                                                                                | 0.02     | 0.02       | 0.04        |
| 65 | 7-Hexadecenol,(Z)                                                                      | 0.02     | -          | -           |
| 66 | 1,5-Dodecadiene                                                                        | 0.01     | -          | -           |
| 67 | α-Terpine                                                                               | -        | 1.26       | 3.00        |
| 68 | 7-Oxabicyclo[4,1,0]heptane, 1-methyl-4-(2-methyloxiranyl)                              | -        | 0.01       | 0.02        |
| 69 | endo-Borneol                                                                           | -        | 0.03       | 0.05        |
| 70 | 2-cyclohexen-1-ol, 1-mrthyl-4-(1-methylethyl)-,trans                                   | -        | 0.02       | 0.02        |
| 71 | α-Copaene                                                                              | -        | 0.02       | -           |
| 72 | Citronellal                                                                            | -        | -          | 0.08        |
| 73 | 1,2,5,9-Tetradecatriene,3,12-diethyl                                                    | -        | -          | -           |
| 74 | Cyclohexene, 1-methyl-4-(1-methylthylidene)                                             | -        | -          | 0.09        |
| 75 | E-7-Tetradecenol                                                                      | -        | -          | 0.08        |
| 76 | Bicycle[2,2,1]heptane-2-one, 1,7,7-trimethyl, -(1S)                                     | -        | -          | 0.04        |
| 77 | Benzaldehyde,4-(methylthyl)                                                             | -        | -          | 0.02        |
| 78 | Carvenone                                                                              | -        | -          | 0.01        |
| 79 | 2-cyclohexen-1-one, 3-methyl-6-(methylthylidene)                                       | -        | -          | 0.02        |
| 80 | α-Cubebene                                                                             | -        | -          | 0.02        |
| 81 | Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylthyl)-,[1S-(1α,7α,8α)]      | -        | -          | 0.03        |
Data in table 1 showed identified black cumin seeds essential oils constituents were analyzed using GC-MS. From the GC-MS result sixty six, sixty and seventy five constituents were identified which constituting 99.8%, 99.27% and 99.17% in Eden, Dershay and Selingo black cumin seeds essential oils. The main constituents of black cumin seeds EO as detected by GC/MS were p-cymene (44.36%), α-thujen (12.57%), trans-4-methoxy thujane (7.39), 9,12-Octadecanoic acid, 9Z,Z- (6.04%), β-pinene (3.04%), α- pinene (2.68), gamma-terpinene (2.50%), cis-vaccenic acid (2.46%), thymoquinone (2.13%), D-limonene (1.83%), phenol-2-methyl-5-(1-methylethyl) (1.60%), bicyclo[3,1,0]hexane,4-methylene-1-(1-methylethyl) (1.52%), terpinen-4-ol (1.13%), cis-4-methoxy thujane (1.06%) in eden variety, p-cymene (45.85%), α-thujen (17.30%), trans-4-methoxy thujane (7.91), β-pinene (4.08%), α- pinene (3.94), gamma-terpinene (3.83%), thymoquinone (2.40%), D-limonene (2.29%), longifolene (1.56%), bicyclo[3,1,0]hexane,4-methylene-1-(1-methylethyl) (1.52%), cis-4-methoxy thujane (1.30%), α-terpinene (1.26%), phenol-2-methyl-5-(1-methylethyl) (1.18%) in dershay variety and and p-cymene (44.31%), α-thujen (13.66%), trans-4-methoxy thujane (8.86%), thymoquinone (3.53%), β-pinene (3.45%), gamma-terpinene (3.24%), α- pinene (3.00), α-Terpinene (3.00), %), phenol-2-methyl-5-(1-methylethyl) (2.56%), D-limonene (2.27%), bicyclo[3,1,0]hexane,4-methylene-1-(1-methylethyl) (1.87%), longifolene (1.56%), 1-Cyclohexene-1-carboxaldehyde (1.53), 2,6,6-trimethyl terpinen-4-ol (1.31%), cis-4-methoxy thujane (1.35%) in variety. The p-cymene and α-Thujane, β-pinene, α- pinene, γ-terpinene, Bicyclo[3,1,0]hexane,4-methylene-1-(1-methylethyl) content of dershy. From Iran black cumin seeds essential oil 32 compounds were identified and the main compounds of the essential oil were trans-anethole (38.3%), p-cymene (38.3%), α-Thujane (14.8%), limonene (4.3%), and carvone (4.0%) Nickavar B. et al., (2003) [17]. The present study revealed that the essential oil isolated from each black cumin variety seeds cultivated in Ethiopia are similar to each other and the others country black cumin essential oil in respect of presence of main compounds. Bahman. N. et al., (2003) [17], Kokoska.L. et al., (2008) [18], Khalid.A.K. et al., (2016) [19].
4. Conclusion
Essential oil content and composition of black cumin affected by types of genetic material, ecological condition and origin of the plant. The result obtained from the study indicates significant variations on black cumin essential oil and chemical composition content from three varieties cultivated in the same agro-ecology. p-cymene (45.85 - 44.31%), α-thujen (17.30 - 12.57%), trans-4-methoxy thujane (8.86 - 7.39%), 9,12-Octadecanoic acid, 9Z,Z)-(6.04% - 0.07%), β-pinene (4.08 - 3.04%), α-pinene (3.94 -2.68%), gamma-terpinene (3.83 - 2.50%), thymoquinone (3.53 - 2.13%), α-terpinene (3.00 – 0. 00%), D-limonene (2.29 - 2.08%), phenol-2-methyl-5-(1-methylethyl) (2.56 -1.52%), cis-vaccenic acid (2.46 - 0.00%), longifolene (1.95 - 1.83%), terpinen-4-ol (0.92 – 1.31%) and cis-4-methoxy thujane (1.35 – 1.06%) are the major constituents of the three varieties of black cumin essential oils cultivated in Ethiopia. The Ethiopian black cumin essential oil contain the required major secondary metabolite for pharmacological and other application.

5. Acknowledgment
The authors would to thank Ethiopian Institute of Agricultural Research (EIAR) for their financial support of this work.

6. Conflict of interest
The authors would like to declare that the study was carried out mainly for academic research purpose without any conflict of interest.

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