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Determination of composition of fatty acid profile of Ethiopian and Indian black cumin oil (*Nigella sativa*)

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Abstract
Aiming at medicinal properties of *Nigella sativa* seeds, this study was performed to determine fatty acid profile of Indian and Ethiopian blackseed oil. Chemical composition (total fatty acid composition) of extracted fixed oil respect to two types of black cumin was investigated by using Gas Chromatography Mass Spectrometry. Evaluated fatty acid profiles of both types of oils show that black cumin oil is rich in polyunsaturated fatty acids where unsaturation is predominant rather than saturation. In Ethiopian fixed oil polyunsaturated fatty acid was 61.39g/100g total fatty acid, Monounsaturated fatty acid 20.94g/100g which is less comparatively and Saturated fatty acid was 14.66g/100g total fatty acid. The main polyunsaturated fatty acid was Linoleic acid (61.25g /100g of total fatty acids) in Ethiopian origin which is higher than that of Indian origin (50.24g/100g of total fatty acid) followed by Oleic acid (17.63g/100g), Palmitic acid (11.36g/100g), Cis-11Eicosenoic acid (3.04g/100g), Stearic acid (2.81g/100g), Meristic acid (0.2g/100g) and Arachidic acid (0.19g/100g) in Ethiopian black seed oil. Respect to Indian type, extracted fixed oil showed 19.09g/100g Oleic acid, 10.83g/100g Palmitic acid, 2.25g/100g Cis-11Eicosenoic acid and 2.47g/100g Stearic acid respectively. The oil was rich in polyunsaturated fatty acid which is more healthy and Ethiopian and Indian *Nigella* seeds or black cumin are good source of oil.

Keywords: black cumin, fixed oil composition, *Nigella sativa*

1. Introduction
Black cumin (*Nigella sativa*) is a member of the Ranunnclaceae family which is an herbal plant native to Mediterranean region. Seeds of black cumin are small in size and angular in shape. They occupy black and dark grey colors. Its initial place of origin is most probably West Asia [1]. A whole black cumin seed can be characterized by a very dark colour and a thin, crescent shape with a pungent bitter taste and smell. A great deal of interest is focused on the oil extracted from this seed either solvent extracted or cold pressing as it is used for medicinal purposes. It has been traditionally used for the treatments related to body functions such as respiratory health, stomach and intestinal health, kidney and liver function, and circulatory and immune system support [2]. Normally Nigella seed oil has a relatively low initial FFA content. During the extraction and pressing procedures, the FFA contents of oils could increase due to heat production. It is reported that oil is extracted by solvent extraction method at 40-60^0 C and even at 70^{0}C. But this hot extraction method can generate some adverse effects in oil properties as well as on constituents having functional, antioxidative and pro oxidative effects [4]. Therefore, most often, the oil extraction is done by a process referred to as cold pressing. Temperatures that are applied to the seeds not higher than 140–176°F (60–80°C) to crush the fat globules and preserve its quality benefits. As quality of the oil is preserved through cold pressing often, this method of extraction is used in present industries. In addition, super critical fluid extraction allows low temperature CO_2 extraction process (up to 104˚F/40˚C) for carrier oils, which is also used in certain industrial applications to ensure a product having a longer shelf life than cold processed carrier oils [3]. Here fatty acid profile is significant in highlighting the quality of the oil. The principal component in the volatile oil of black cumin is p-cymene. it has been traditionally used for the treatments for asthma, cough, bronchitis, headache, rheumatism, fever, influenza and eczema [4]. When consider about utilization of blackseed in Sri Lanka, local companies import black cumin from countries like India and Ethiopia. Those imported black seeds undergo through cold pressing to extract oil. Treated oil is taken for various productions with the aim of curing diseases such as cholesterol, diabetes and even in weight loosing. In some local products, black seed oil is incorporated for value addition. Although a large market share is not occupied by Sri Lanka in the production of black seed oil, nowadays, there is an increasing interest in healthier and nutritional products in local market. Therefore, black cumin oil is established as a valuable oil used by many customers worldwide. Hence the focus of this study is to determine fatty acid profile of Ethiopian and Indian black cumin seed oil as to emphasize the health properties.

2. Materials and Method
2.1 Plant Material
Nigella sativa seeds were collected from a well-known Sri Lankan company which imports one type from India and other one from Ethiopia. Black cumin samples were separately grinded in a heavy grinder for 2 minutes, to pass 1mm diameter aperture and samples were preserved in dry stoppered containers at -20^{0}C until analysis. (A.O.A.C 17th edition 2000, Official Method 920.164 Preparation of Test sample)

2.2 Sample preparation for Fatty acid methyl esters (FAMEs)

Oil was extracted in the Soxhelt apparatus by using pet ether as the solvent and 1g was measured after evaporating the solvent completely. Then 3ml of benzene/toluene and 1.5ml of sodium methoxide were added and mixed well. After adding 4.5 ml of methanol, solution was kept on hot plate at 50^0C for 15 minutes. After cooling to room temperature, 10 ml of distilled water followed by 9 ml of hexane were added while mixing well. Mixture was allowed to stand for about 10 minutes to separate water & hexane layers. After the separation, upper hexane layer was transferred from a dropper to test tube which containing sodium sulphate anhydrous to remove any traces of moisture. Finally, it was transferred to a capped & labeled glass vial for GC analysis.

2.3 Determination of fatty acids through GC-MS

FAMEs of fatty acids were identified by Gas Chromatograph (Model 7890 A, Agilent technologies) equipped with Mass Spectrometer (Model 5975 C inert XL EI/CI MSD) with triple axis detector. Used carrier gas was Helium at flow rate of 1ml/min. FAMEs were separated by using a polar capillary column RTX 5, 0.32 mm internal diameter, 30m length & 0.25 µm film thickness (Restex Corp., Bellefonte, PA, USA). Injected volume of sample was 1µL at temperature of 270^0C. The initial column temperature was100^0C programmed by 20^0C /min until 170^0C (hold time 0 min) & finally 5^0C/min until 280^0C (hold time 16.5 min). The total run time of sample was about 45 minutes. The percentage of fatty acid was calculated as the ratio of the partial area to the total peak area of FAMEs.

3. Results and discussion

Methanol extracts of black seeds were analyzed by Gas Chromatography Mass Spectrophotometer. There are many techniques employed to identify the phytochemicals from plant extract of Nigella sativa and GC-MS is such reliable method to identify fatty acid profile and functional compounds. Fixed oil of Nigella sativa was subjected to analyze by GCMS and chromatogram obtained by GC-MS exhibited number of peaks at various retention times and intensities. Thereafter compounds relevant to these peaks were identified on the basis of molecular weight. Identified fatty acid composition respect to Indian and Ethiopian origins are given in table 1 and 2

Table 1: Fatty acid composition of fixed oil of Ethiopian origin *Nigella sativa*

Fatty acid composition summery	Results of Ethiopian origin (g/100g)
Polyunsaturated fatty acid	61.39
Monounsaturated fatty acid	20.94
Saturated fatty acid	14.66
Trans fatty acid	<0.01
Fatty acid composition in detail	(prominent)
Linoleic acid	61.25
Oleic acid	17.63
Palmitic acid	11.36
Cis-11Eicosenoic acid	3.04
Stearic acid	2.81
Meristic acid	0.2
Arachidic acid	0.19

Table 2: Fatty acid composition of fixed oil of Indian origin *Nigella sativa*

Fatty acid composition	Results of Indian origin (g/100g)
Linoleic acid	50.24
Oleic acid	19.09
Palmitic acid	10.83
Cis-11Eicosenoic acid	2.25
Stearic acid	2.47

As shown in the table 1 and 2, Ethiopian origin black seed oil has polyunsaturated fatty acid (PUFA) content of 61.39g /100g total fatty acids and 50.24g/ 100g total fatty acids in Indian origin blackseed oil. Both PUFA contents are comparable to that in the cold-pressed *Nigella* oil recorded in Lutterodt *et al*., (2010) [5]. Polyunsaturated fatty acids can exhibit healthier properties as Omega 3 is identified as a healthier fatty acid.

Ethiopian Black Cumin Seed Oil contained significant level of monounsaturated fatty acids (MUFA) content 20.94g/100g total fatty acids and it is in accordance with values recorded in Lutterodt *et al*., (2010) [5]. Furthermore, saturated fatty acid composition (14.66g/100g of oil) of the extracted oil is less compared to unsaturated fatty acids respect to both types of oils and the present investigation is similar to literature values mentioned in Nergiz et at.,(1993)[6]; Nickavar *et al*., (2003)[7].

The primary fatty acid in the cold-pressed Ethiopian origin black cumin seed oil (BCSO) was linoleic acid (18:2n-6) at a level of 61.25 g/100 g total fatty acids and followed by oleic acid (18:1n-9) at a level of 20.94g/100g and Palmitic (16:0) acid at 11.3 g/100 g total fatty acids, respectively as shown in the table 4.1. Similarly, as shown in the table 4.2 Indian type shows primarily, linoleic acid (18:2n-6) at a level of 50.24 g/100 g total fatty acids and followed by oleic acid (18:1n-9) at a level of 19.09 g/100g and Palmitic (16:0) acid at 10.83g/100 g total fatty acids, respectively. According to these results, except for oleic fatty acid other primary fatty acids like linoleic acid, Palmitic, stearic fatty acid contents are higher in Ethiopian origin black cumin oil than Indian origin black cumin oil thus implies (PUFA) content in Ethiopian is higher than Indian type. Furthermore, these result of fatty acid composition in detail are accordance with records in Sultan *et al*., (2009) [8].

4. Conclusion

Black cumin is a revealed medicinal plant still commonly used as black seed oil in food and pharmaceutical industry. Both Indian and Ethiopian black cumin oils are consisting rich fatty acid profiles. Healthier properties of the oil which were revealed make interesting path ways to research in future studies.

5. References

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