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# Comparative chemical profiling and antimicrobial activity of *Nigella sativa* seeds oils obtained from different sources

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**ABSTRACT:** *Nigella sativa* L. seeds are widely used in Sudan as a spice, food preservative, and medicine. Sudan does not grow the plant. The study aimed to compare the chemical profile and antibacterial activity of fixed and essential oils of *N. sativa* from Ethiopian and Indian seeds and the oil offered by Attarin in the local market. A Soxhlet device extracted fixed oils and hydrodistillation to obtain essential oils and analysed their oil profile using GC-MS. Disk diffusion was used to test antimicrobial activity. The fixed oil of Ethiopian (EFO) and Indian (IFO) seeds contained ten components, with linoleic acid (50.12% in EFO and 57.69% in IFO) being the most abundant. Ethiopian seeds were used to extract the essential oil. 51.96% of the oil was hydrogenated monoterpenes. The main chemicals were p-cymene (36.76%) and thymoquinone (18.70%). There were fixed and essential oils in the Attarin oil sample. The main component was linoleic acid (14.61%), followed by p-cymene (13.85%). The maximum antibacterial activity (MIC 6.25 $\mu$g/disc) was seen in both fixed and liquid oil samples against *Escherichia coli* and *Pseudomonas aeruginosa*. The best anti-*P. aeruginosa* action was attarin oil (MIC 12.5 $\mu$g/disc). Finally, the Sudanese market needs to standardise *N. sativa* seeds and oil.

## 1. INTRODUCTION

*Nigella sativa* L. (family Ranunculaceae), sometimes known as black cumin, has been used as a spice and to treat a variety of diseases in many places of the world since ancient times. The seeds are currently used in a variety of culinary, medicinal, and cosmetic purposes (Hosseinia et al., 2019). The seeds are mostly used in Sudan as a spice, to protect food from pathogenic and spoilage germs, and in traditional medicine to alleviate articulation pain, stomachache, jaundice, diabetes, headaches, and hypertension (Ghazali et al., 1994; Issa et al., 2018). Currently, it is believed that the seeds can be used to treat coronavirus. *N. sativa*, on the other hand, is not farmed in Sudan, and seeds are primarily imported from Ethiopia and India.

The seed and its oils were found to exhibit several pharmacological properties among them, anticancer, antiviral, antibacterial, antipyretic, galactagogue, carminative, antidiabetic, and antioxidant activities (Abdelfadil et al., 2013; Darakhshan, Pour, et al., 2015; Darakhshan, Tahvilian, et al., 2015; Salem, 2005; Salomi et al., 1992). Most of these biological activities were mainly associated with thymoquinone, which is the major component of the oil, in addition to polyunsaturated fatty acids, phenols, quinone, carvacrol and 4-terpineol (A. Ahmad et al., 2013; I. Ahmad et al., 2013; Darakhshan, Pour, et al., 2015; Khader & Eckl, 2014; Kooti et al., 2016; Manju et al., 2016; Tavakkoli, Ahmadi, et al., 2017; Tavakkoli, Mahdian, et al., 2017). Silva et al. (2020) found that other volatile monoterpenes such as -thujene, cymene, -pynene, and 3-carene were responsible for *N. sativa* oil's enzyme inhibitory capacity against acetylcholinesterase and 3-hydroxi-3-methylglutaryl-coenzyme A (HMGR), as well as its anticancer impact.

In Sudan, consumers obtain herbs and herbal mixtures for therapeutic purposes from so-called 'Attarin' stores. Almost all of these preparations, including oils, were not manufactured industrially, and their traditional methods of preparation are poorly documented. Additionally, there is no thorough analytical technique for standardising the various herbal medications accessible in Sudan. Thus, the purpose of this study was to ascertain the chemical diversity in fixed and volatile oils extracted from *N. sativa* seeds from Ethiopia and India, as well as to evaluate the quality of an oil sample sold at an Attarin shop. Additionally, the antibacterial activity of these oils was assessed.

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## 2. MATERIALS AND METHODS

### 2.1. Plant materials

*N. sativa* seeds from Ethiopia and India were purchased from the primary supplier in Khartoum, Sudan. The seed oil sample was obtained from the Attarin shop in Khartoum's local market.

### 2.2. Preparation of oils

Separately, the dried Ethiopian and Indian seeds were mashed using a pestle and mortar. Around 25 g of seeds were extracted with n-hexane in a Soxhlet device for 4 hours and concentrated under reduced pressure to produce the fixed oil. The essential oil was extracted using the hydrodistillation process, which involved immersing 500 g of seeds powder in 3 L of water for 2.5 hours using a Clevenger-type. Over anhydrous sodium sulphate, the essential oil was dried. Until used, all oils samples were stored at 4 °C in amber-colored vials.

### 2.3. Preparation of fatty acid methyl ester

Methyl esters derivatization of fixed oils samples was prepared as described by Tobergte and Curtis (2013).

### 2.4. Gas chromatography-mass spectrometry

Essential and fixed oils were analyzed using a gas chromatography-mass spectrometry system (GC-MS) as described by Hemmati and coworkers (2020).

### 2.5. Antimicrobial activity

Antimicrobial activity of oils samples was evaluated by the method described by Zaidan et al. (2005). Antibacterial activity was performed against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Antifungal activity was carried out against the clinical isolate *Candida albicans*. Ampicillin, Gentamicin and Fluconazole were used as positive controls and dimethyl sulfoxide (DMSO) as the negative control.

## 3. RESULTS AND DISCUSSION

### 3.1. Chemical profile of oils samples

Fixed oils were extracted from *N. sativa* seeds grown in Ethiopia and India. The Ethiopian fixed oil (EFO) was obtained as a light yellow-coloured oil while that of Indian origin (IFO) had a light brown colour. Both samples gave more or less the same percentage yield of fixed oil (EFO = 38.25% and IFO =38.05%) (Table 1). These amounts were higher than those reported for fixed oil obtained from seeds grown in Turkey (24.4–29.5 % (Nimet et al., 2015)) and Iran ((30–35 %) (Harzallah et al., 2012; Hosseinia et al., 2019)). They were comparable to yields reported from seeds grown in Yemen (36.8–38.4% (Al-Naqeeb et al., 2009) and Morocco (37%, (Gharby et al., 2015).

Fatty acids profiles of the EFO and IFO are presented in Table 2. Ten compounds were identified in each oil and in general, the two samples shared common compounds (7) with variable relative abundance. The dominant fatty acids in both oils were linoleic ((omega 6)) (EFO = 50.12% and IFO = 57.69%) and oleic (EFO = 27.76% and IFO =24.91%) acids respectively. Palmitic (EFO =13.23% and IFO = 9.71%) and stearic (EFO = 3.61% and IFO = 2.48%) acids were also found in considerable amounts in both samples. This was in agreement with fixed oil obtained from seeds grown in other geographical regions (D'antuono et al., 2002; Ghahramanloo et al., 2017; Şener et al., 1985). The amount of linoleic acid was higher in IFO while that of oleic acid was relatively higher in the EFO. Linoleic acid content was comparable or slightly higher than recorded from Iranian (51.67% and 55.95%) (Hosseinia et al., 2019) and Saudi-Arabia (56.5%) (Gharby et al., 2015) seeds samples and lower than that of Tunisian origin (58%) (Harzallah et al., 2012).

**Table 1**
Extraction yield and colour of oils from seeds of *Nigella sativa*.

| Sample | Extraction yield (%) | Colour |
|---|---|---|
| Ethiopian fixed oil | 38.25 | Light yellow |
| Indian fixed oil | 38.05 | Light brown |
| Ethiopian essential oil | 9.2 | Dark brown |
| Indian essential oil | - | Dark brown |
| Attarin oil | - | Dark brown |

**Table 2**
Chemical composition of Ethiopian and Indian fixed oil samples of *Nigella sativa* seeds.

| RT | Compound name | Area (%) | |
|---|---|---|---|
| | | Ethiopian | Indian |
| 15.089,15.090 | Palmitic acid, C16:0 | 13.23 | 9.71 |
| 16.072 | Margaric acid, C17:0 | - | 0.06 |
| 16.740, 16.759 | Linoleic acid, C19:2 | 50.12 | 57.69 |
| 16.782, 16.799 | Oleic acid, C19:1 | 27.76 | 24.91 |
| 17.001, 17.004 | Stearic acid, C18:0 | 3.61 | 2.48 |
| 17.221 | 11,14-Eicosadienoic acid, C21:2 | 2.00 | - |
| 17.246, 17.382 | Erucic acid, C22:0 | 0.72 | 0.40 |
| 17.345 | Linoleic acid, C19:2 | - | 0.93 |
| 18.522, 18.525 | cis-11,14-Eicosadienoic acid, C21:2 | 2.10 | 1.93 |
| 18.753, 18.757 | Arachidic acid, C20:0 | 0.23 | 0.12 |
| 20.371 | Behenic acid, C22:0 | 0.19 | - |
| 20.568 | Z-6,17-Octadecadien-1-ol acetate, C20:2 | - | 1.18 |
| 21.872 | Lignoceric acid, C24:0 | 0.04 | - |
| | Total | 100 | 99.41 |

Hydrodistillation of *N. sativa* seeds of Ethiopian origin (EEO) resulted in a yield of 9.2% dark brown-coloured essential oil (Table 1). Chemical profile of EEO is presented in Table 3.

**Table 3**
Chemical composition of Ethiopian essential oil sample of *Nigella sativa* seeds.

| RT | Compound name | Area (%) |
|---|---|---|
| 3.035 | α-Phellandrene | 3.91 |
| 3.124 | 1R-α-Pinene | 0.84 |
| 3.236 | Benzene, (2-methylpropyl)- | 0.19 |
| 3.555 | Sabinene | 0.58 |
| 3.612 | β-Pinene | 1.91 |
| 4.066 | (+)-2-Carene | 0.75 |
| 4.183 | β-Cymene | 36.76 |
| 4.220 | D-Limonene | 2.27 |
| 5.091 | trans-4-methoxy thujane | 1.32 |
| 5.408 | cis-Carveol | 12.86 |
| 6.255 | Terpinen-4-ol | 5.17 |
| 7.264 | Thymoquinone | 18.70 |
| 7.699 | Acetic acid, bornyl ester | 0.40 |
| 8.085 | Phenol, 2,3,5,6-tetramethyl- | 1.62 |
| 8.600 | Cedrene | 1.50 |
| 8.891 | α-Himachalene | 0.08 |
| 9.305 | γ-Gurjunene | 0.15 |
| 9.363 | Longifolene | 9.32 |
| 9.511 | Aromandendrene | 0.09 |
| 10.816 | delta-Amorphene | 0.07 |
| 11.640 | β-Cadinene | 0.30 |
| 12.677 | α-Bisabolol | 0.27 |
| 15.504 | 2,5-bornanediol | 0.13 |
| 15.677 | Nerolidol | 0.20 |
| 16.739 | Linolelaidic acid, methyl ester | 0.06 |
| 16.777 | 10-Octadecenoic acid, methyl ester | 0.06 |
| 16.850 | 9-Undecenal, 2,10-dimethyl- | 0.44 |
| 17.246 | 6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl- | 0.05 |
| | Total | 100 |
| | Hydrogenated monoterpenes | 51.96 |
| | Oxygenated monoterpenes | 38.38 |
| | Hydrogenated sesquiterpenes | 11.21 |
| | Oxygenated sesquiterpenes | 0.52 |

28 compounds were identified. Hydrogenated monoterpenes represented 51.96% of the oil followed by oxygenated monoterpenes (38.38%) and hydrogenated sesquiterpenes (11.21%) respectively. p-Cymene (36.76%) represented the major compound followed by thymoquinone (18.70%), cis-carveol (12.86%), longifolene (9.32%), terpinen-4-ol (5.17%) and α-phellandrene (3.91%) respectively. Generally, these compounds were also identified from essential oil of *N. sativa* grown in many geographical regions (Ghahramanloo et al., 2017; Gharby et al., 2015; Harzallah et al., 2012; Nimet et al., 2015). However the major difference was in the percentage amount of these components which were mainly influenced by many factors like source of the plant, different environmental factors and agronomic techniques used (D'antuono et al., 2002; Kokoska et al., 2008; Nickavar et al., 2003). Moreover, the method of extraction determined the percentage amount of these compounds. For example Kokoska et al. (2008) found that essential oil of *N. sativa* extracted by hydrodistillation and dry steam distillation was dominated by p-cymene, while thymoquinone was found to be the major compound from the supercritical fluid extraction.

Application of hydrodistillation technique did not extract any detectable amount of essential oil from *N. sativa* seeds of Indian origin. Many factors were suggested to explain this observation; among them, as suggested by Johnson, the pH of water is often reduced during distillation and consequently some constituents of essential oils, which might be present in the Indian sample, like esters may be hydrolyzed while others like acyclic monoterpene hydrocarbons and aldehydes undergo polymerization (Johnson, 2007). It was also proposed that oxygenated compounds like phenols have the tendency to dissolve in still water and thus hinder their complete removal by distillation (Johnson, 2007). Chemical profile of the Attarin oil sample is presented in Table 4. Thirty fve compounds were identified and the oil was mainly composed of fatty acids and their derivatives in addition to terpenes. Linoleic acid (14.61%) was the dominant compound followed by p -cymene (13.85%), (Z)6,(Z)9-pentadecadien-1-ol (13.52%), Z,Z-8,10-hexadecadien-1-ol (11.02%), thymoquinone (10.62%) and α-phellandrene (7.64%) respectively. Also sterols were identified with γ-sitosterol representing 2.19% of the oil. Thus, it could be suggested that the Attarin oil sample was a mixture of fixed and essential oil of *N. sativa* seeds.

### 3.2. Antimicrobial activity of oils samples

The antibacterial and antifungal activities of all *N. sativa* seeds oils were evaluated against tested microorganisms by determination of MIC values. Results are presented in Table 5. The four oils samples showed variable antimicrobial activity. Both EFO and IFO strongly inhibited the two Gram negative bacteria *E. coli* and *P. aeruginosa* with MIC value of 6.25 μg/disc. Also IFO displayed highest antibacterial activity (MIC = 6.25 μg/disc) against *B. subtilis* while the EFO exerted the best activity (MIC = 25 μg/disc) against *S. aureus*. Interestingly, the Attarin oil sample exhibited considerable antimicrobial activity against tested bacteria with the highest activity against *P. aeruginosa* (MIC = 12.5 μg/disc). Concerning the antifungal activity against the fungus *C. albicans*, all oil samples exhibited lower inhibitory effect (MIC = 100 μg/dis) on comparison with their antibacterial activity. Ampicillin, Gentamicin and Fluconazole were used as positive control and DMSO as negative control.

However, it has been demonstrated that the general antibacterial activity of *N. sativa* seeds was more related to its essential oil (Hasan et al., 1989; Rathee et al., 1982). Thymoquinone was found to strongly contribute into the antibacterial and antifungal activities of the essential oil (Aljabre et al., 2005; Kokoska et al., 2008). Moreover, Zheng et al. (2005) demonstrated that unsaturated fatty acids especially linoleic acid possessed remarkable antibacterial activity by inhibiting fatty acid synthesis. Also the fatty acid alcohol (Z)6,(Z)9-pentadecadien-1-ol was reported to possess antibacterial activity. However, in the present study the two fixed oils (EFO and IFO) gave higher antibacterial activity than the EEO.

**Table 4**
Chemical composition of Attarin oil sample of *Nigella sativa* seeds

| RT | Compound name | Area (%) |
|---|---|---|
| 3.036 | α-Phellandrene | 7.64 |
| 3.125 | 1R-α-Pinene | 1.67 |
| 3.556 | Sabinen | 0.49 |
| 3.612 | β-Pinene | 1.39 |
| 4.177 | p-Cymene | 13.85 |
| 5.092 | trans-4-methoxy thujane | 0.31 |
| 5.408 | cis-Carvotanacetol | 2.04 |
| 7.277 | Thymoquinone | 10.62 |
| 8.600 | Cedrene | 0.20 |
| 9.359 | α-Himachalene | 0.86 |
| 15.046 | 9-Borabicyclo[3.3.1]nonane, 9-(1-methylbutyl) | 0.08 |
| 15.505 | Sandaracopimar-15-en-8.beta.-yl acetate | 0.23 |
| 15.554 | Pentadecanoic acid | 0.30 |
| 15.685 | Citronellyl butyrate | 0.31 |
| 16.732 | 9,12-Octadecadienoic acid | 0.71 |
| 16.772 | 10-Octadecenoic acid, methyl ester | 0.44 |
| 16.845 | 9-Undecenal, 2,10-dimethyl- | 3.97 |
| 17.279 | Linoleic acid | 14.61 |
| 18.146 | Glycerol 1-palmitate | 0.13 |
| 18.391 | Nerolidyl acetate | 0.25 |
| 18.532 | 1,2-Dipalmitoyl-rac-glycerol | 1.80 |
| 19.611 | Linoleoyl chloride | 1.98 |
| 19.822 | 2-Methylundecanal | 0.55 |
| 19.986 | (Z)6,(Z)9-Pentadecadien-1-ol | 13.52 |
| 20.139 | 2,2,4,7-Tetramethyl-3,6,9-trioxa-2-siladecane | 0.32 |
| 20.191 | Glyceryl 1,3-distearate | 0.28 |
| 20.564 | Z,E-7,11-Hexadecadien-1-yl acetate | 0.15 |
| 21.343 | Imidazole, 2-amino-5-[(2-carboxy)vinyl]- | 1.71 |
| 21.470 | 11,14-Eicosadienoic acid, trimethylsilyl ester | 3.78 |
| 21.706 | Z,Z-8,10-Hexadecadien-1-ol | 11.02 |
| 22.579 | Squalene | 0.39 |
| 25.753 | Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)- | 0.24 |
| 26.028 | Stigmasterol | 0.71 |
| 26.587 | γ-Sitosterol | 2.19 |
| | Total | 98.74 |
| | Fatty acids and their derivatives | 49.04 |
| | Hydrogenated monoterpenes | 25.04 |
| | Oxygenated monoterpenes | 12.66 |
| | Hydrogenated sesquiterpenes | 0.86 |
| | Oxygenated sesquiterpenes | 13.52 |

This could partly explain the relatively low antibacterial activity of the Attarin oil sample which was a mixture of fixed and essential oil. Overall, results of antimicrobial activity of *N. sativa* seeds oils give support to its traditional uses in treating infectious diseases caused by the tested microorganisms. Moreover, it is clear that, beside the source of sample, extraction yield, chemical composition and antimicrobial activity of *N. sativa* seeds are largely associated to the extraction technique. Thus, selection of appropriate technique and extraction preparation should be well considered in standardization of herbal drugs.

**Table 5**
Antimicrobial activity of oil samples of *Nigella sativa* seeds.

| Oil samples | MIC (μg/disc) | | | | |
|---|---|---|---|---|---|
| | *B. subtilis* | *S. aureus* | *E. coli* | *P. aerugi-nosa* | *C. albicans* |
| Ethiopian fixed oil | 25 | 25 | 6.25 | 6.25 | 100 |
| Indian fixed oil | 6.25 | 100 | 6.25 | 6.25 | 100 |
| Ethiopian essential oil | 100 | 100 | 25 | 100 | 100 |
| Attain oil sample | 25 | 100 | 25 | 12.5 | 100 |
| Ampicillin | 4 | 4 | - | - | - |
| Gentamicin | - | - | 4 | 4 | - |
| Fluconazole | - | - | - | - | 8 |
| DMSO | - | - | - | - | - |

## 4. CONCLUSION

The present study revealed that the main fatty acid in both fixed oils was linoleic acid. Essential oil was only obtained from the seeds of Ethiopian origin and was dominated by hydrogenated monoterpenes. p-Cymene followed by thymoquinone were the major compounds. Therefore, from the results obtained in this work, it was clear that the major variance between Ethiopian and Indian *N. sativa* seeds was in the essential oil which was not extracted from the later by hydrodistillation technique. Accordingly it was suggested that other technique of extraction could be performed to extract the volatile oil from the Indian sample. Also from the obtained results, Attarin oil sample was suggested to be composed of a mixture of fixed and essential oils. It showed considerable antimicrobial activity, although less effective than the fixed oil samples towards the tested two Gram negative bacteria. Nevertheless, it is very important to standardize the seeds and oil of *N. sativa* available on the Sudan market and particularly to consider a method of extraction for essential oil for the Indian seeds sample.

## CONFLICTS OF INTEREST

Authors state no conflict of interest for this study.

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## AUTHOR CONTRIBUTIONS

MS, SMY - Research concept and design; FT - Collection and/or assembly of data; FT, SMY - Data analysis and interpretation; FT, SMY - Writing the article; MS, SMY - Critical revision of the article; MS, SMY - Final approval of the

article.

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