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Identification of Essential Oil Components from *Nigella sativa* Seed by Gas Chromatography-mass Spectroscopy

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Abstract: The volatile oils from the seed of *Nigella sativa* were obtained by steam distillation. Gas chromatography-mass spectrometry was used to identify the components. Nine volatile oils were identified and 2-methyl-5(1-methyl ethyl)-Bicyclo[3.1.0]hex-2-ene was the major constituent (62.28%) while alpha-pinene was the minor (2.28%).

Key words: Volatile oils, *Nigella sativa*, steam distillation, extraction

INTRODUCTION

Essential oils are oily liquids which are entirely, or almost entirely, volatile without decomposition, such as oil of anise, are solid at 15.5°C, but melt to form liquid at slightly higher temperature, Denston (1939). The essential oils have characteristic fragrances and tastes and are mixtures of known and unknown compounds. They may contain hydrocarbons, terpene alcohols, aldehydes, ketones, phenols and esters.

Nigella sativa belongs to the family *Ranunculaceae* and is called *Habbatus sauda* in Hausa. It is an annual herbaceous plant and is believed to be indigenous to the Mediterranean region but now it has been cultivated into other parts of the world including Africa (Mozoffarin, 1998; Zargari, 1990). According to Amin (1991), the seeds are believed to have galactagogue, carminative, laxative and antiparasitic properties. An antibacterial effect of the phenolics fraction of the seed oil was first reported by Topozada *et al*. (1965). Latter, the diethyl ether extract of *Nigella sativa* was also reported by Hanafi and Hatem (1991) to inhibit the growth of bacteria. Aqueous suspension of *Nigella sativa* seeds was reported to have an analgesic effect comparable to aspirin test on rat (Randhawa and Al-Ghandhi, 2002).

The antischisommicidal effect against *Schistosoma mansoni* and antimalarial activities have been studied (Azza *et al*., 2005; Abdulelah and Zainal-Abidin, 2007).

The chemical constituents of the fixed and volatile oils of *Nigella sativa* obtained from Iran have been reported by Nickavar *et al*. (2003). The study indicates the total fatty acid composition and some volatile components.

This study is aimed to describe the chemical composition of volatile oil of *Nigella sativa* seeds obtained from Maiduguri extracted by steam distillation method.

MATERIALS AND METHODS

Collection of seeds: The seeds of *Nigella sativa* were obtained from Monday market in Maiduguri, Borno state and authenticated by malam H.U. Dukku of Biological sciences programmed, Abubakar Tafawa Balewa University, Bauchi. The seeds were crushed and ground to fine powder. The powdered sample was stored in clean and dry place until used.

Extraction of the volatile oils: Steam distillation method was employed in the extraction process. 400 g of finely powdered sample was placed in a distillation flask connected to a steam distiller, condenser and a receiver and was hydro distilled for four hours. The distillate was extracted with dichloromethane. The dichloromethane layer was separated and dried over anhydrous magnesium sulphate and filtered. The filtrate was concentrated by distilling off the solvent in a rotary evaporator. The percentage yield was calculated and the oil was subjected to Thin Layer Chromatography (TLC), Infra Red (IR) and Gas-mass Spectrometry (GC-MS) analysis.

Analysis of the volatile oil: The GC-MS analysis was performed with a quadruple GC-MS system, Agilent GC model 6890N and Agilent mass selective detector, 5973 series; capillary column (30 m x 0.25 mm; 0.25 μm film thickness). The carrier gas was helium and column head pressure of 15 psi yielding a linear flow rate of 0.8 m/min. The split ratio was 1: 10 and the initial column temperature was held at 200°C for 15 min and then raised at 10°C/min and maintained at 260°C until all components had eluted. The components were identified by matching their mass spectra in the Wiley 275 library and their retention indices were compared with literature values.

RESULTS AND DISCUSSION

The seed of *Nigella sativa* was subjected to steam distillation and extracted with dichloromethane, dried over magnesium sulphate and the yield was 0.4%. The thin layer chromatogram of the components shows the

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Table 1: Infra Red (IR) spectral data of volatile oil from *Nigella sativa*

Group	Intensity	Description	Wave number cm^{-1}	Extract
-OH (hydrogen bonded)	Medium	Broad absorption	3460	Volatile oil extract
C-H stretching	Strong	Sharp absorption	2900	
C=O stretching	Strong	Sharp absorption	1650	
C=C	Weak	Sharp absorption	1598	
C-O	Strong	Sharp absorption	1210	

Table 2: Chemical composition of the volatile oil of *Nigella sativa*

Compound	P	RT (min)	MW	M/Z (% intensity of fragments
2-methyl-5(1-methyl ethyl)-Bicyclo[3.1.0]hex-2-ene	62.28	5.305	136	136(20), 121(10), 105(10), 93(100), 91(60), 79(40), 65(5), 39(10)
Alpha-pinene	2.28	5.000	136	136(13), 121(20), 93(100), 91(42), 77(25), 67(10), 53(10),41(10)
Beta-pinene	2.49	6.527	136	136(14), 121(15), 93(100), 91(27), 79(25), 69(26), 53(8), 41(24),
1-methyl-2-(1-methyl ethyl)benzene	45.70	7.725	134	134(27),119(100), 93(4), 91(22), 63(5), 39(4)
4-methyl -1-(1-methyl ethyl)-3- cyclohexen-1-ol	4.80	10.89	154	154(41), 136(25), 139(5), 111(80), 93(79), 121(5), 95(10), 69(4), 43(30), 41(24)
2-methyl-5-(1-methyl ethyl)-2,5-cyclohexadiene-1,4-dione	30.8	12.302	164	164(68), 136(48), 121(54), 108(8), 93(40)
2-methyl-5-(1-methyl ethyl)phenol	4.45	13.175	150	150(35), 135(98), 91(10),65(3), 39(30)
2,6,6,9-tetramethyl tricyclo[5.4.0.$0^{(2,8)}$]undec-9-ene	2.49	13.709	204	204(25), 189(8), 133(48), 119(100), 105(50),91(30), 69(6), 41(10)
Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	4.20	9.163	204	204(40), 189(50), 175(20), 161(100), 147(30), 133(50), 119(50), 105(55), 91(60), 79(50), 41(55)

P = Percentage; RT = Retention time; MW = Molecular weight

following Rf values: 0.07, 0.21, 0.33, 0.42, 0.60, 0.80 and 0.90 by using dichloromethane and petroleum ether of ratio 1:1.

The IR values are shown in Table 1. The absorption band at 3460 cm^{-1} revealed the presence of OH group. The stretching absorption band at 1650 cm^{-1} may be due to carbonyl absorption, while the absorption band at 2900 cm^{-1} revealed C-H stretching.

The mass spectral data for the volatile oil from *Nigella sativa* is presented in Table 2. Nine components were identified as seen in Table 2.

The oil consists of three monoterpenoid hydrocarbons (2-methyl-5-(1-methyl ethyl) bicyclo[3.1.0]hex-2-ene, alpha-pinene and beta-pinene), one phenyl hydrocarbon (1-methyl-2-(1-methylethyl)benzene), one phenol hydrocarbon (2-methyl-5-(1-methyl ethyl)phenol), one monoterpenoid alcohol (4-methyl -1-(1-methyl ethyl)-3-cyclohexen-1-ol), two sesquiterpenoid hydrocarbons (2,6,6,9-tetramethyl tricyclo[5.4.0.$0^{(2,8)}$]undec-9-ene and Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene) and one monoterpenoid ketones (thymoquinone or 2-methyl-5-(1-methyl ethyl)-2,5-cyclohexadiene-1,4-dione).

Volatile oils have been found to exhibit antioxidant activity for example, the volatile oils studied by Lado *et al.* (2004) showed that the reducing capacities of the components were lower than the values obtained for volatile oils.

Conclusion: The ingredients obtained from this study indicate that the oil can be fully utilized for the manufacture of perfumery products, antimicrobial and antiseptic agents.

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