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GC-MS Analysis of *Nigella sativa* Seeds and Antimicrobial Activity of its Volatile oil

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ABSTRACT

The present study dealt with the hydro distillation of Nigella sativa seeds and GC-MS analysis. The total composition of the oil was 86.7%. The seed volatile oil was tested against 19 microbes (Gram positive, Gram negative and fungi), which showed that Gram negative bacteria Haemophilus influenza, Klebsiella pnemoniae, and Proteus vulgaris were highly sensitive against the antimicrobial agent, whereas the fungi such as Trichoderma vibriae,. Pencillium rubrum; and Dermatophyte: Trichophyton mentagrophytes; had no response at the 20µl concentration.

Key words: *Nigella sativa,* Volatile oil, Antimicrobial activity, Antifungal activity

INTRODUCTION

The role of the volatile oils is predominant in many industries, particularly in the pharmaceutical, clinical and food industries. The oil and its constituents are well documented as antimicrobial agents (Knobloch et al., 1989, Pepeljnjak et al., 2003). The volatile oils are complex mixtures of the compounds which mainly having monoterpines, sesquiterpines hydrocarbons with general formula $(C_5H_8)_n$ (Svoboda and Hampson, 1999). These oils act in monitoring the blood circulation, nerve growth, liver, and cholesterol etc (Vardharajan, 1985). It also possesses antimicrobial activity. Some Plants like *Nigella sativa*, *Cuminum Cyminum*, *Papaver somniferum*, etc. have been considered as a protective agent against carcinogenesis (Aruna and Sivaramakrishnan, 1996, Hailat, 1995). The oil from *N.sativa* is used in preliminary clinical medicine for cough and bronchial asthma (Vardharajan, 1985). The seeds are considered as carminative stimulant, diuretic, emenagogue, galactagogue and are used in the treatment of mild cases of puerperal fever (Vardharajan, 1985, Hailat, 1995).

N.sativa is an annual herb and is widely spread in southern Europe and western Asia, abundantly in Mediterranean region (Vardharajan, 1985). In India it is found in Punjab, Bihar, Assam and Himachal Pradesh (Vardharajan, 1985). The seeds are trigonous black rugulose tubercular and used as a flavouring agent and for the medicinal purposes (Vardharajan, 1985, Hailat, 1995).

In view of its medicinal value the *N.sativa* was chosen for the extraction of essential oil with an aim to establish its antimicrobial activity against

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Gram-positive and Gram-negative bacteria and fungi.

MATERIALS AND METHODS

The seeds of *N.sativa* were procured from local market and were fine powered and hydro-distilled at 100^0C in a Clevenger apparatus (Haborne, 1984). The volatile oil was collected, dried over anhydrous sodium sulphate, stored in brown bottles and finally kept in refrigerator for further GC-MS analysis.

GC-MS Analysis

A Shimadzu 17A GC coupled with Shimadzu QP5050 A (quadruple) Mass Spectrometer (Shimadzu, Japan), equipped with EI and a fused silica column DB-5 (30m x 0.25 mm i.d.) of 0.25μm film thickness was needed. The oven temperature at 50^0C for 5 minutes and then programmed from 50-280^0C for 40 minutes. Helium flow rate of 2ml/min, with the split ratio of 1:30 mode was used for sample injection of 1μl and ionization voltage of MS-analysis was run by EI technique at 70ev. The volatile oil constituents were identified by matching their MS and retention index data with those of the standards ethnic spectra and by matching their fragmentation pattern in Mass Spectra with those of WILEY 139.LIB and NIST 12.LIB (3) The retention indices were calculated by Kovats's procedure (Masada, 1976, Adams, 1989).

Antimicrobial susceptibility test

The antimicrobial activity was determined by the disc diffusion method using the Kirby-Bauer method (Bauer and Kirby, 1966). The discs of 6 mm diameter were prepared with Whatman No 1 filter paper. The volatile oil of concentration 20μg for the test was applied to the discs. Inoculum was prepared with the fresh cultures of bacterial strains, which were grown in tryptic-soy agar for 18h at 37 ± 1^0C with physiological saline, 3 x 10^6 cells ml^{-1}. Inoculum density was compared with Mac-Farlands standard solution of $BaSO_4$ (0.1ml of 1% $BaCl_2$ + 9.9ml of 1% H_2SO_4). The cultures were cultivated on Sabouraud dextrose agar with addition of 50mg/l Chloramphenicol (sigma, Germany) for 5 days for the yeasts and 10 days for fungi and dermatophytes at 25±5ml of Muller Hington agar for the bacterial strains, and the same amount of inoculum was cultured Sabouraud agar for the fungi. Then the agar was inoculated with the culture and incubated at room temperature for 25 minutes. The discs were arranged on the surface of the inoculated agar plates and pressed gently to adhere to the surface of the agar. The plates were incubated for 24-48h at 35-37^0C. After incubation, the diameter of the zone of inhibition was measured.

RESULTS AND DISCUSSION

The GC-MS analysis of the *N. sativa* volatile oil showed 31 compounds (Table-1), which included two new chemical compounds viz. 2(1H)-Naphthalenone ($C_{11}H_{18}O$) and Uvdin ($C_{15}H_{24}O_3$). The percentage of total compounds was 86.7%.

The results of the antimicrobial activity of the *N. sativa* volatile oil were presented in Table-2 and compared with the standard and accordingly, the efficacy of volatile oil was far better than the standard. Further, from the data found in Table-2, the order of sensitivity of microorganisms was Gram-negative bacteria followed by Gram-positive bacteria, Yeast and Dermatophyte, but at 20mg of volatile oil, the *Trichophyton mentagrophytes*, *tricoderma vibriae*, *penicillium rubrum* were found to be resistant to the volatile oil. The presence of biological active compounds such as α-thujene, 2(1H)-naphthalenone, α-pinene, α-phellandrene, limonene, thymoquinone, myristicin etc in *N.sativa* volatile oil contributed the antimicrobial activity of volatile oil.

Table 1 - Chemical composition of *Nigella sativa* volatile oil constituents.

Compound	Percentage	Compound	Percentage
α-Thujene	2.4	*p*-Cymene-8-ol	0.4
3-Methyl Nonane	0.6	Nerol	1.3
α-Pinene	1.2	Estragole	1.9
Sabinene	1.4	Dihydrocarvone	0.3
β-Pinene	1.3	Carvone	2.0
Myrcene	0.6	Thymoquinone	11.8
n-Decane	0.4	Anisaldehyde	1.7
α-Phellandrene	0.6	Trans-Anethole	27.1
p-Cymene	9.0	Carvacrol	3.7
Limonene	4.3	α-Longipinene	0.3
1-Methyl-3-propyl benzene	0.7	*n*-Tetradane	0.2
γ-Terpinene	0.5	Longifolene	5.7
1-Ethyl-2,3-dimethyl benzene	0.2	Uvidine	1.3
2(1H)-Naphthalenone	2.6	Myristicin	1.4
Fenchone	1.1	*n*-Hexadecane	0.2
Terpinen-4-ol	0.7	Apiole	1.0
Total	27.6		60.3

Table:2 - Antimicrobial activity of the *Nigella sativa* volatile oil by Disc diffusion method.

	Microorganisms	Inhibition Zone in mm	
		Oil disc (20µg)	Standard
Gram-Positive Bacterial species	*Staphylococcus aureus* MTCC 737	18	25
	Streptococcus pneumoniae MFBF	14	nt
	Bacillus subtilus MTCC 121	10	nt
	Micrococcus luteus MTCC 1541	11	20
Gram-Negative Bacterial species	*Pseudomonas aeruginosa* MTCC 1688	14	11
	E.Coli MTCC 1687	14	17
	Vibrio cholerae MFBF	16	nt
	Salmonella typhi MFBF	10	nt
	Proteus vulgaris MTCC 1771	12	12
	Haemophilus influenzae MFBF	32	nt
	Neisseria gonorrhoeae MFBF	18	nt
	Klebsiella pneumoniae MFBF	16	16
Yeast	*Candida albicans* MTCC 184	08	12
Fungi	*Aspergillus niger* MTCC 1344	10	13
	Aspergillus flavus MFBF	09	14
	Tricoderma vibriae MFBF	0	16
	Penicillium rubrum MFBF	0	16
	Chaetomium globosum MFBF	07	12
Dermatophyte	*Trichophyton mentagrophytes* MFBF	00	12

nt=not tested., Clindamycin 2mg/ml for S.aureus, Gentamicin 2mg/ml for *Ps. aeruginosa, Proteus vulgaris*; Tetracycline 3mg/mlE.Coli and *B.subtilus* Clotrimazole 5mg/ml for *C. albicans*; Nystain 10mg/ml for *A.niger* and *T.mentagrophytes*.
MFBF: number of strains from the collection of microorganisms of the Dept. of Microbiology and biotechnology, Anantapur. MTCC: Microbial type culture collection centre.

CONCLUSIONS

The presence of rich biological active compounds in *N.sativa* volatile oil, which contributed antimicrobial properties, has highlighted the plant as good medicinal plant.

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