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Chemical composition and antidermatophytic activity of *Nigella sativa* essential oil

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The essential oil of *Nigella sativa* extracted by standard hydrodistillation method from Cleavenger's apparatus was done for Gas Chromatography (GC) and Gas Chromatography-Magnetic Separation (GC-MS) analysis. The oil yield was 0.35% (v/w) and had the presence of 17 volatile components representing 97.54% of the total oil which were identified. The major components of essential oil were para-cymene (54.13%), alpha-thujene (10.43%), hydrocarveol (10.40%), longifolene (6.97%), beta-pinene (3.10%), and other components were present in trace amounts. The *in vitro* antimicrobial activity against five pathogenic fungi was done by disc diffusion method and microdilution method. The oil **represents the strong antimicrobial activity against *Microsporum gypseum, Trichophyton rubrum and Trichophyton simii* with diameter of inhibition zone and activity index 38 mm (AI: 1.90), 20 mm (AI: 1.33) and 35 mm (AI: 1.09), respectively as compared with *Chrysosporium tropicum* with diameter of inhibition zone 26 mm (AI: 0.86) and *Chrysosporium evolceanui* with diameter of inhibition zone 25 mm (AI: 0.71).**

Key words: Essential oil, antimicrobial, microdilution, dermatophytes.

INTRODUCTION

Nigella sativa (Family Ranunculaceae) is a spice which is well known for its medicinal properties. It has been extensively in use for centuries in folk medicines, both as herb and for oil by people in Asia, Middle East, and Africa for medicinal purposes. Seeds are used as a new source of edible oils and food applications as spice and condiments in cakes, breads, pastries, curries, pickles and in seasoning etc. The seeds contain 40% fixed oil, a saponin (melantin), and up to 1.4% volatile oil (Chevallier, 1996).

The seeds of *N. sativa* have been used traditionally for centuries in the Middle East, Northern Africa and South Asia for the treatment of various diseases (Brutis and Bucar, 2000; Gilani et al., 2004).

Black seed oil has been shown to be effective against a wide spectrum of organisms-bacteria like *Bacillus cereus, Bacillus subtilis, Bacillus pumilus,* S*taphylococcus aureus*, *Escherichia coli*, *Salmonella abony* (Gurdip et al., 2005; Damjanova et al., 2002), pathogenic yeast like *Candida albicans* etc. A few antimicrobial work of the volatile oil of the seeds of *N. sativa* has been reported (Toppozada et al., 1965; Taha et al., 1975; Agarwal et al., 1979; Hanafy and Hatem, 1991; Hasan et al., 1989). Essential oils of the seeds are reported to be used for both allergic and irritant contact dermatitis as herbal remedies (Steinmann et al., 1997). The aim of this study is to describe the detailed chemical composition of volatile oil of *N. sativa* seeds and antimicrobial activity of the volatile oil against different pathogenic microorganism: *Chrysosporium tropicum, Trichophyton simii, Trichophyton rubrum, Microsporum gypseum, Chrysosporium evolceanui.*

MATERIALS AND METHODS

Extraction and preparation of oil

Nigella sativa seeds were purchased from local market of Jaipur, and extraction of oil from *N. sativa* seeds was carried out by standard hydrodistillation method from Cleavenger's apparatus, and

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all operation were carried out at room temperature. The crushed seed powder (200 g) was placed in a separate flask together with distilled water (1 L). After 5 to 6 h, oil was collected from the apparatus and it was dehydrated by passing through anhydrous sodium sulphate for removal of water traces stored into dark bottle and at 4°C until use, yield was 0.35% (w/v). Essential oil was used for disc diffusion test and determination of minimum inhibitory concentration (MIC) and also for GC/GC-MS analysis.

Gas chromatography (GC)

GC was carried out using a Shimadzu GC-2010 equipped with a flame ionization detector (FID) on a capillary column (Omega SPTm, 30 m × 0.25 mm ID). The column temperature was programmed from 80 to 230°C at a rate of 2°C/min and held at 230°C. The injector and detector temperature were 270 and 280°C, respectively. The flow rate of the carrier gas nitrogen (N_2) was 1.21 ml/min.

Gas chromatography and mass spectroscopy (GC-MS)

The essential oil sample was analyzed by GC-MS-QP 2010 plus (Shimadzu company) operating at 70 eV ionization energy. Capillary column (Omega SPTm, 30 m × 0.25 mm i.d.) with nitrogen gas was used as the carrier gas with split ratio 80.0. The column temperature was programmed from 80 to 230°C at a rate of 2°C/min and held at 230°C. The injector and detector temperature were 270 and 280°C, respectively. The flow rate of the carrier gas helium was 1.21 ml/min. The components of the standard and essential oil sample were identified by comparison of their mass spectra and retention time with those given in literature, and by comparison with the mass spectra of the Wiley library or with the published mass spectra.

Screening of antifungal activity of essential oil

C. tropicum, T. simii, T. rubrum, M. gypseum, and *C. evolceanui* fungi were isolated from the soil samples collected from different localities of Jaipur district by To-Ka-Va hair baiting technique (Vanbreuseghem, 1952), maintained on Sabourauds dextrose agar (SDA) media, and identified by microscopy and various biochemical tests. Oil was screened for their antifungal activity against these pathogenic fungi by disc diffusion method (Rios et al., 1998). Standard size Whatman No.1 filter paper discs, 6.0 mm in diameter, sterilized by dry heat at 140°C in an oven for one hour were used to determine antifungal activity. SDA medium for disc diffusion test was prepared. After sterilization, it was poured into sterilized petri plates and allowed to solidify. The spore suspension of each of the fungi was prepared from 8 to 10-day-old cultures separately. A suspension that was just turbid (~0.5 McFarland standard), equivalent to 1 to 5 × 10^6 CFU/ml prepared by suspending the selected fungi in 0.9% NaCl solution, vortexing and homogeneous suspension, was used for inoculation. The suspension was vortexed and 0.1 aliquots were spread over the respective agar medium plates.

Sterilized filter paper disc were soaked in neat essential oil. An oil-saturated disc of 100 µl concentration per disc was placed on an agar plates containing fungal spore suspension. Similarly, solutions of standard antibiotics (Gentamycin (Sigma) and Ketoconazole (Sigma) of 10 mcg/disc concentration) for antifungal activity were prepared and impregnated in the filter paper discs. These discs were then placed over the plates preceded with respective microorganisms. The plates were incubated at 30°C for 48 to 72 h. Three replicates were kept in each case and average values were calculated. The diameter of the inhibition zones was measured in mm and the activity index was calculated on the basis of the size of the inhibition zone. The activity of oil was measured by the following formula:

Activity Index = Inhibition zone of sample / Inhibition zone of standard

Statistical analysis

Data were presented as mean ± standard error of the mean (SEM).

Determination of minimum inhibitory concentration using microdilution method

The modified microdilution method of Provine and Hadley (2002) was followed to determine MIC. Media used for MIC was semisolid agar media. Brain heart infusion agar aliquots of semisolid agar media (Bactoagar; Difco Laboratories) at a pH of approximately 7.4 were poured into a 16 × 125 mm glass tubes and autoclaved. A suspension that was just turbid (~0.5 McFarland standard), equivalent to 1 to 5 ×10^6 CFU/ml, prepared by suspending the selected fungi in 0.9% NaCl solution, vortexing and homogeneous suspension, was used for inoculation. Different concentrations of diluted essential oil (75% acetone + 25% essential oil) were added in media containing test-tubes, afterwards a standard platinum loopful (~0.001 ml, Himedia, Flexiloop) of the inoculum suspension was inserted deep into each tube of medium containing a different concentration of oil, as well as an oil-free control, by a centered down-up motion to form a two dimensional inoculum. The tubes were then incubated at 37°C for 48 to 72 h to determine the MIC. MIC was read to be the lowest concentration at which there was no visible growth of the organism. Then, by visual inspection, good growth of the respective fungi in oil-free medium as a control was detected (after 48 h for filamentous fungi) afterwards, the growth in all tubes at different concentrations of oils was compared with that of the oil-free control in order to determine inhibition after 48 to 72 h of incubation.

RESULTS

A clear, yellowish colored oil was obtained from the seeds of *N.sativa* with 0.35% (v/w) yield. GC-MS analysis revealed the presence of 17 volatile components, representing 97.54% of the total oil which were identified (Figures 1 and 2). The percentage composition and names of the essential oil components are listed in Table 1. The major components of essential oil were para-cymene (54.13%), alpha-thujene (10.43%), dihydrocarveol (10.40%), longifolene (6.97%), beta-pinene (3.10%), limonene (2.21%), 4-terpinenol (1.82%), alpha-longipinene (1.57%), isothujol (1.46%), carvacrol (1.16%), sebinene (1.13%) and other components were present in trace amounts as presented in Table 1.

In Disc diffusion method (Table 2), *N. sativa* exhibited excellent antidermatophytic activity against selected fungi. Maximum zone of inhibition was found to be 38 mm against *M. gypseum* (inhibition zone (IZ): 38 mm, activity index (AI): 1.90) as compared to standard drug ketoconazole (20 mm) and gentamycin (no inhibition or 6 mm). Likewise, *N. sativa* also showed excellent activity against *T. rubrum* (IZ: 30 mm, AI: 1.33) and *T. simii* (IZ:

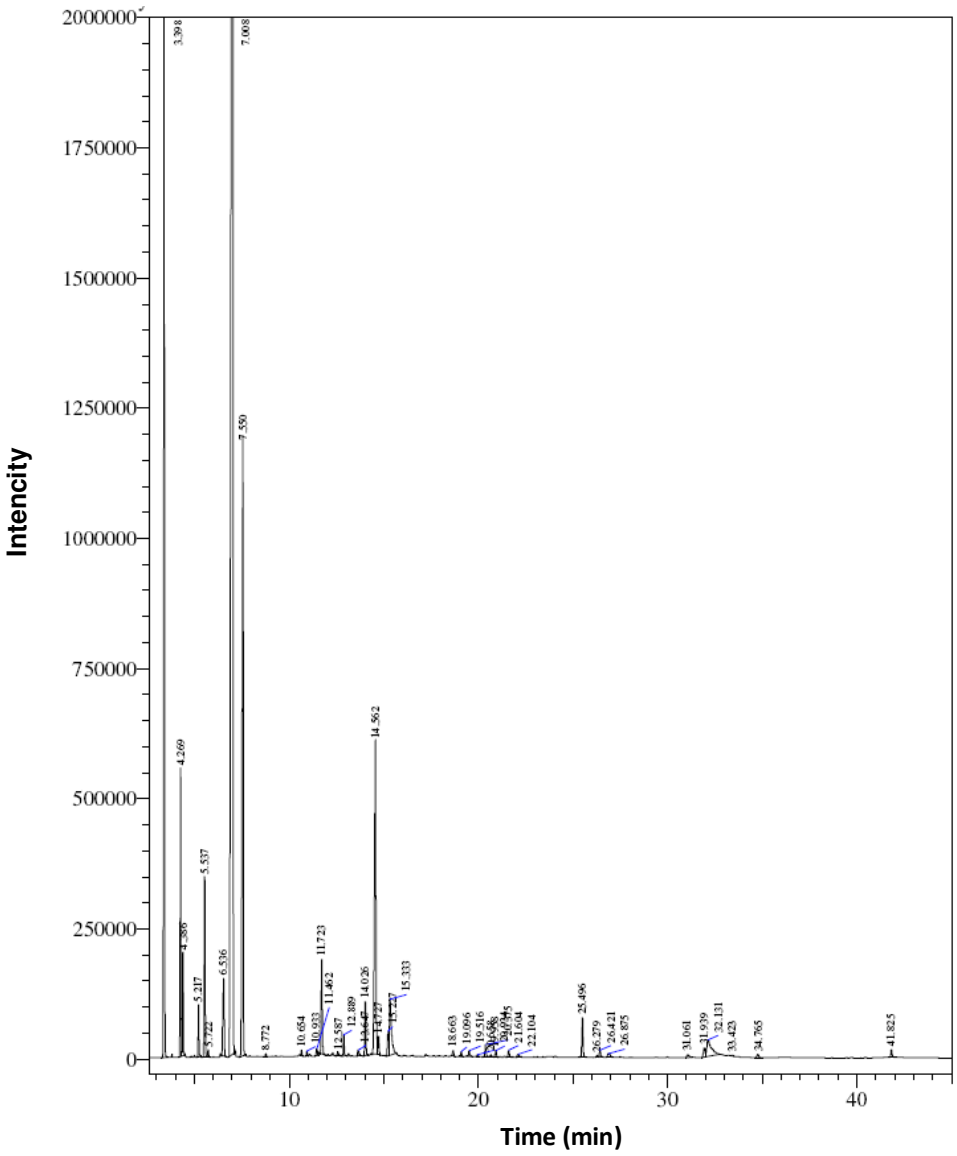

Figure 1. Gas chromatogram of essential oil of *Nigella sativa*.

35 mm, AI: 1.09) as compared to standard. Zone of inhibition against *C. tropicum* (IZ: 26 mm, AI: 0.86) and C. *evolceanui* (IZ: 25 mm, AI: 0.71) was slightly comparable to standard drug ketoconazole 30 and 35 mm, respectively. Ketaconazole is a best antifungal standard against dermatophytes. Tables 3 and 4 represent MIC of essential oil by microdilution method against different pathogenic organisms. The results showed (Table 3) that neat essential oil (100% concentration) exhibited inhibitory action at < 0.1 µl/ml against *C. tropicum, T. rubrum, M. gypseum* and *T. simii* but *C. evolceanui* showed inhibitory action at 0.4 µl/ml as MIC value. For further investigation, we diluted the oil at 25% concentration and checked against *C. tropicum, T. rubrum, M. gypseum* and *T. simii.* The results (Table 4) showed that diluted essential l oil (25% concentration) exhibited inhibitory action at 0.7 µl/ml against *C. tropicum,* 0.5 µl/ml against *T. rubrum* and *M. gypseum*, 0.8 µl/ml against *T. simii* as MIC value. In the present investigation, *C. evolceanui* was found to be more susceptible fungus at 100% concentration of oil whereas rests of four fungi were found to be resistant.

DISCUSSION

Spices and herbs have been used for many years by different cultures to enhance the flavor and aroma of foods, in preserving foods and for their medicinal value. According to World Health Organization (Santos et al., 1995), medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should

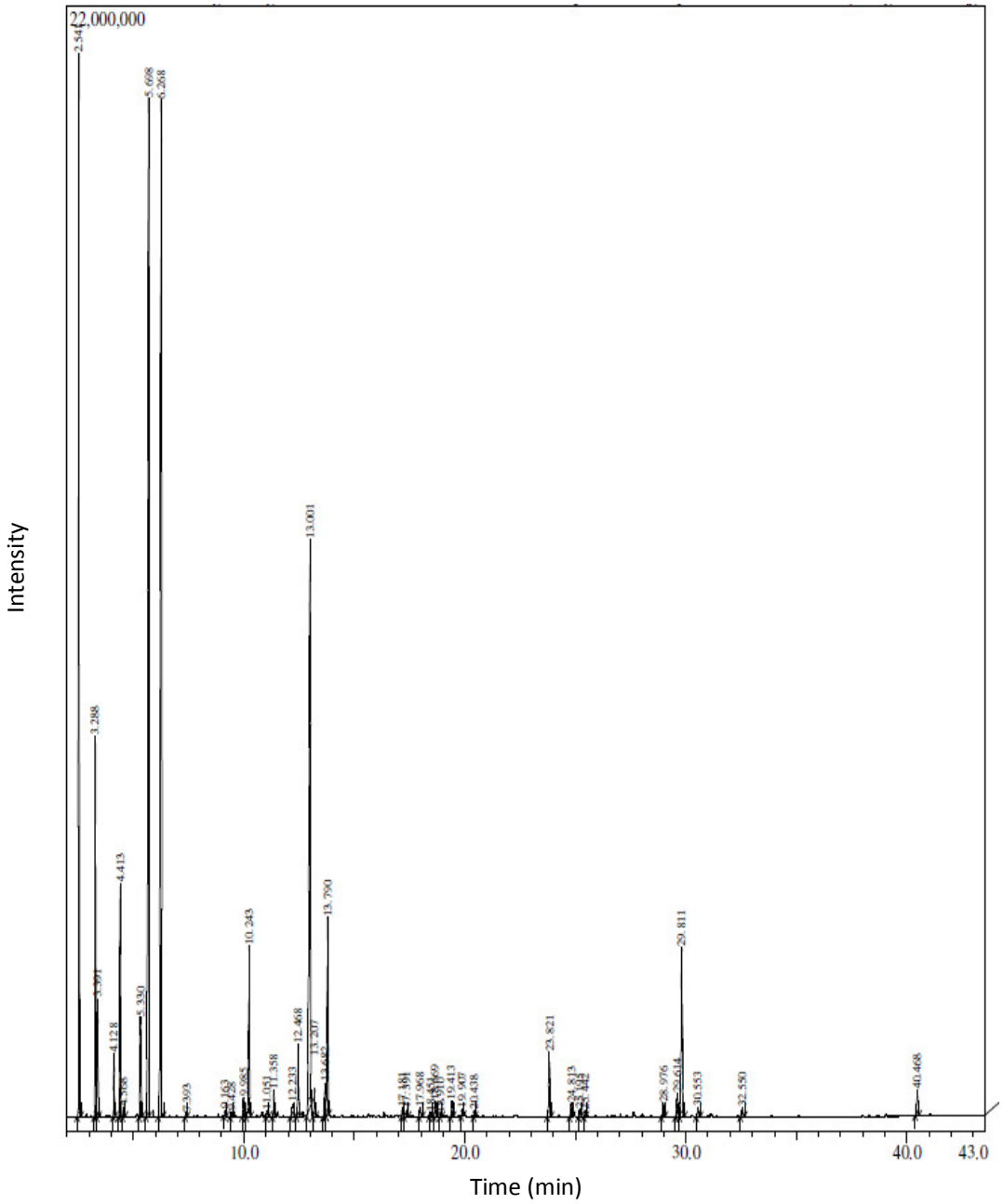

Figure 2. Chromatogram of essential oil of *Nigella sativa* by GC-MS.

be investigated to better understand their properties, safety and efficiency (Ellof, 1998; Nascimento et al., 2000; Mothana et al., 2009).

In our result, the major components of essential oil were para-cymene (54.13%), alpha-thujene (10.43%), dihydrocarveol (10.40%), longifolene (6.97%), beta-pinene (3.10%) and other components were present in trace amounts as presented in Table 1. Present study showed the similarity with Harzallah et al. (2011). They investigated the chemical composition by gas chromatography with electron impact mass spectrometry (GC–EIMS), the antibacterial and the cytotoxic activities of Tunisian *N. sativa* essential oil. The major one was p-cymene (49.48%) whereas thymoquinone represented only 0.79%. The essential oil (2.43 mg/disc) containing only 3.35 µg of thymoquinone showed pronounced dose dependant antibacterial activity against *Streptococcus mitis, Streptococcus mutans, Streptococcus constellatus* and *Gemella haemolysans* (15.5 ± 0.707 mm). Their MIC values are representatives of a good effect. So, the essential oil strongest activity was seen against *S. mitis, S. mutans, S. constellatus* and *G. haemolysans* (MIC 2.13 mg/ml). The most important antibacterial activity was seen against *S. constellatus* (MIC 4 µg/ml).

Table 1. Compounds identified from essential oil of *Nigella sativa* by GC/GC-MS.

Peak number	Reaction time	Area (%)	Name of compound
1	3.39	10.43	Alpha-Thujene
2	4.26	3.10	Beta-pinene
3	4.38	1.13	Sabinene
4	5.21	0.62	Alpha-terpinene
5	5.53	2.21	Limonene
6	6.53	1.46	Isothujol
7	7.00	54.13	Para-cymene
8	7.55	10.40	Dihydrocarveol
9	11.72	1.57	Alpha-longipinene
10	12.88	0.31	Limonene oxide
11	14.02	0.84	Cis-limonene oxide
12	14.56	6.97	Longifolene
13	14.72	0.31	Isobornyl acetate
14	15.22	0.35	Beta-caryophyllene
15	15.33	1.82	4-Terpinenol
16	25.49	0.73	Caryophyllene oxide
17	32.131	1.16	Carvacrol
Total		97.54	

Table 2. Antifungal activity of *N. sativa* essential oil against pathogenic organisms.

Essential oil	Test strain	IZ of Sample	IZ of Ketoconazole	AI of Ketoconazole
	C. tropicum	26±0.57	30±0.57	0.86
	T. rubrum	20±1.73	15±1.15	1.33
N.sativa	*T. simii*	35±1.15	32±0.57	1.09
	M. gypseum	38±1.52	20±1.73	1.90
	C. evolveceanui	25±1.15	35±1.15	0.71

Concentration of oil used 100%. IZ = inhibition zone (in mm) including the diameter of disc (6 mm), AI = activity index.

Table 3. MIC of *N. sativa* essential oil (Neat) against different pathogenic organisms.

Essential oil	*C. tropicum*	*T. rubrum*	*T. simii*	*M. gypseum*	*C. evolveceanui*
N. sativa (µl/ml)	<0.1	<0.1	<0.1	<0.1	0.4

Likewise, Singh et al. (2005) reported that *N. sativa* essential oil showed complete growth inhibition against *B. cereus, B. subtilis* and *S. aureus, Pseudomonas aeruginosa* at 2,000 and 3000 ppm, respectively, by the agar well diffusion method. GC/GC-MS studies on the essential oil of *N. sativa* resulted in the identification of 38 components representing 84.65% of the total amount. The major component was p-cymene (36.2%) followed by thymoquinone (11.27%), α-thujene (10.03%), longifolene (6.32%), β-pinene (3.78%), α-pinene (3.33%) and carvacrol (2.12%).

Similarly, Toma et al. (2010) studied the qualitative and quantitative analysis of *N. sativa* essential oil using GC-MS method. *N. sativa* oil represents the presence of 30 compounds among them: alpha-pinene (13.75%), limonene (2.55%), para-cymene (43.58%), carvacrol (2.53%) and thymoquinone (1.65%). These results are also in agreement with the results of other investigations (Mozaffari et al., 2000; Nickavara et al., 2003), although the essential oil composition depends on the used extraction technique, instrument conditions, season of sample collection etc.

The present results of antimicrobial activity of *N. sativa* essential oil by microdilution and disc diffusion methods showed the agreement with Zuridah et al. (2008). They reported that *N. sativa* as a source of antidermatophytes

Table 4. MIC of diluted *N. sativa* essential oil (25% concentration) against different pathogenic organisms.

Concentrations of *N. sativa* oil (µl/ml)	Growth visually inspected in different concentrations of oil (%)			
	C. tropicum	*T. rubrum*	*T. simii*	*M. gypseum*
0.1	100	100	100	75
0.2	75	75	100	50
0.3	75	50	75	50
0.4	50	25	50	25
0.5	25	0	50	0
0.6	25	0	25	0
0.7	0	0	25	0
0.8	0	0	0	0
0.9	0	0	0	0
1.0	0	0	0	0
Control without oil	100	100	100	100

drugs could be used for treatment of fungal skin infection caused by *T. rubrum T. interdigitale, T. mentagrophytes, Epidermophyton floccosum*, *Microsporum canis* etc. The minimum inhibitory concentration of the oil was between 50 and 400 µl/ml for the various strains, and demonstrated the therapeutic potential of the seed oil for the treatment of diarrhoea. Likewise, Ara et al. (2005) studied the *in vitro* antimicrobial activity of the volatile oil of *N. sativa* seeds against fifteen pathogenic microbial strains including Gram +ve , Gram -ve bacteria and yeast (Candida Albicans). The oil showed strong sensitivity to all the organisms. The zone of inhibition was found to be 13 to 32 mm, at a dose of 600 µg/disc. MIC was also determined against *S. aureus* American Type Culture Collection (ATCC) and *E. coli* ATCC was found to be 187 and 375 µg/ml, respectively. Similarly, Islam et al. (1989) tested the antifungal activity of volatile oil of *N. sativa* seeds against twenty fungi including pathogenic and industrial fungi. The oil in 100 mcL/disc showed very strong inhibition (40 mm) against five fungi, moderate (20 to 39 mm) against eight fungi and mild inhibition (20 mm) against six fungi, and no zone of inhibition against one fungus. Oil was also tested for MIC value against three pathogenic fungi; *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger*. Minimum value (< 0.50mc/disc) was recorded against *A. fumigates*

The present findings are also in agreement with the observation of Uzma et al. (2008) who reported the anti-fungal activity of essential oils extracted from the seeds of neem (*Azadirachta indica*), mustard (*Brassica campestris*), black cumin (*N. sativa*) and asafoetida (*Ferula assafoetida*) against eight seed borne fungi: *A. niger, A. flavus, Fusarium oxysporum, Fusarium moniliforme, Fusarium nivale, Fusarium semitectum, Drechslera hawiinesis* and *Alternaria alternata.*

Thus, in the present study, the essential oil possess higher antifungal activity against pathogenic fungi and can be used to cure dermatophytic infections and may potentiate the efficacy of chemotherapeutics and may have a role as a herbal, traditional medicine, and pharmaceutical for the treatment of fungal infections. Further study should be followed to isolate the pure component of essential oil and testing, as antimicrobial agents for specific antimicrobial effect.

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