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Antimicrobial Activity of Four *Nigella* Species Grown in Southern Turkey

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ABSTRACT

Antimicrobial activity of chloroform, acetone and methanol extract of four *Nigella* species flower, stem, leaves and seeds was investigated by disk diffusion method. Plant species were collected from Ahir Mountain in Kahramanmaras, Turkey which is a hotspot of biodiversity ecologically. Among the plant part of *Nigella* sp., a significant antimicrobial activity was obtained by acetonic extraction of *Nigella sativa* seeds. Even if the antimicrobial activity spectrum considered against tested microorganism, acetonic extract of *N. sativa* seeds was the most effective on both Gram negative and Gram positive bacteria and least effective against *K. Pneumonia*. Chloroform and methanol extract of *N. arvensis* and *N. unguicularis* were the least effective against these microorganisms. This study further verified the antimicrobial properties and comparison of *Nigella* sp. as a potential antimicrobial agent.

Key Words: *Nigella* sp.; Antimicrobial; Antibacterial;Seed; Acetonic Extract

INTRODUCTION

Interest in herbal medicine among the people dates back to ancient times in many countries. *Nigella* sp. is known as black cumin, is used for culinary and medicinal purpose in Middle Eastern countries. Genus *Nigella* comprises about 15 species of annual plants in the family *Ranunculaceae*. The *Nigella* sp. seeds are consumed and processed in Southern Europe, Rusia, North Africa, Sudan, Etiopia, Kenya, Syria, Iran and Afganistan (Tutin *et al*., 1964). *Nigella* sp. seeds contain 36-38% fixed oils, protein, alkaloids, saponin and 0.4-2.5% essential oils (Hosseinzadeh *et al*., 2007). Fatty acid determination analysis showed that the seed oils composed of oleic acid (39-44.5%), linoleic acid (36-37.6%), palmitic acid (6.31%), myristic acid (0.26%), stearic acid (2.45%) and linolenic acid (1.9%). The analysis of essential oils extracted by steam distillation mainly contained nigellon, 2-methyl-4-isopropyl-p-quinone and thymoquinone (Aktuzlu, 1976). Four alkaloids have been reported as constituents of *N. sativa* seeds. Nigellicine and nigellidine have an indazole nucleus, whereas nigellimine and its N-oxide are isoquinolines (Ali & Blunden, 2003).

Beyond antibiotic resistance research for infection control, scientists are increasingly investigating the antimicrobial properties of plant extracts and fractions (Taskova *et al*., 2002; Kariba, 2002; Malika *et al*., 2004; Imelouane *et al*., 2009). Several scientific reports have described the inhibitory effect of plants on a variety of microorganisms, although considerable variation for resistance of different microorganisms to a given plant and of the same microorganisms to different plants has been observed (Arora & Kaur, 1999). Although the antimicrobial activities of *Nigella sativa* extract has been reported (Hanafy & Hatem, 1991; Morsi, 2000), this report deals with the antimicrobial activities of four different *Nigella* species (*N. damascena, N. sativa, N. arvensis, N. unguicularis*).

MATERIALS AND METHODS

***Nigella* samples.** *Nigella* sp. (*N. damascena, N. sativa, N. arvensis, N. unguicularis*) samples were collected from Kucukgol region (1400 m) of Ahir Mountain, at 37° 37'43'' N and 36°54'59''E in Kahramanmaras, Turkey. This mountain is one of the most important centres of endemism and hotspot of biodiversity ecologically. The specimens were identified mainly by using Flora of Turkey (Davis, 1982) in University of Sutcu Imam.

Microorganisms. The microorganisms used in this study were *Escherichia coli* DM, *Enterobacter aerogenes* CCM 2531, *Klebsiella pneumoniae* FMC 5, *Bacillus cereus* FMC 19, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Pseudomonas aeruginosa* DSM 50071, *Staphylococcus aureus* Cowan 1, *Listeria monocytogenes* Scoot A, *Mycobacterium smegmatis* RUT, *M. luteus*, *Proteus vulgaris* and *Saccharomyces cerevisiae* UGA 102, *Rhodotorula rubra*, *Candida albicans* and *Kluvyeromyces fragilis* obtained from culture collection of Department of

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Extraction. After identification of *Nigella* specimens, plant parts to be investigated were grounded in an omnimixer and extracted for 24 h in a Soxhlet extractor with 150 mL of chloroform (Ilçim *et al*., 1997; Dulger *et al*., 1997). The sample was concentrated in a rotary evaporator at 50-55°C. After 3 mL chloroform addition, the sample was stored -50°C until loaded on the antibiotic discs (Bagcı & Dıgrak, 1996). The preparation of antibotic disc was accomplished by loading 25 µL of the sample to the sterile discs (Schleicher & Schül, Nr. 2668, Germany). Chloroform, aceton and methanol loaded discs were also used as control.

Determination of antimicrobial activity. The antimicrobial effects were tested by the disc-diffusion method. Mueller Hinton agar plates were cultured with a standardized inoculums (10^8 cfu mL^{-1}) of each bacterial strain and also Sabouraud dextrose agar were cultured with each of yeast strains (2.1 x 10^3 cfu mL^{-1}) (Collins *et al*., 1989). The plates were incubated at 35-37°C for 24 h and 30°C for 48 h for bacterial strain and fungal strain, respectively. After incubation the diameter of zone of inhibition was measured by a compass.

RESULTS AND DISCUSSION

With the disc diffusion method antimicrobial effect of *Nigella* sp. extracts produced different inhibiton zone on tested microorganisms. Among the *Nigella* species' leaves used, Chloroform extract of *N. damascena* showed the moderate antimicrobial activity compared to control antibiotic ampicillin (Table I). When the stem extract considered the highest antimicrobial activity was obtained by chloroform extract of *N. unguicularis*. *Bacillus megaterium* was the most sensitive microorganism to this extract among the others (Table II). Acetonic extract of capsule of *N. unguicularis* showed the highest inhibitory activity on tested microorganisms (Table IV). Chloroform extract of flowers and capsules of *N. arvensis* showed the better antimicrobial activity compared to other extracts (Table III). Among the plant part of *Nigella* species, the significant antimicrobial activity was obtained by acetonic extraction of *Nigella sativa* seeds. Even if the antimicrobial activity spectrum considered against tested microorganism, *N. sativa* seeds were the most effective part of the plant (Table V).

The antimicrobial activity of *Nigella* seeds in this study are in confirmity with the results of Hanafy and Hatem (1991), Morsi (2000) and Chaudhry and Tariq (2008). The acetonic extract was effective both gram negative and gram positive bacteria. On the other hand, chloroform and methanol extract of *N. arvensis* and *N. unguicularis* were the least effective against the microorganisms. Of all the microorganisms, *B. cereus*, *S. aureus* and *P. aeruginosa* were the most sensitive to chloroform extract of *N. unguicularis* stem and similarly *B. Subtilis, B. cereus B. megaterium* and *L. monocytogenes* were the most sensitive to chloroform extract of *N. arvensis* capsules. While *B. megaterium* was the most sensitive to chloroform extract of *N. arvensis* capsule. *K. Pneumoniae* was the most sensitive strain to acetonic extract of *N. sativa* seeds (Table I-V).

While considering the chemicals used for extraction, chloroform and acetone were the effective solvent in showing better inhibition against the microorganism. However, methanol indicated more resistance, which is probably due to the reason that methanol did not remove the antimicrobic agents from the samples (Senhaji *et al*., 2005).

Table I. Antimicrobial Activity of Chloroform, Acetone and Methanol extract of *Nigella* sp. leaves.

Microorganims	*N. damascena*			*N. sativa*			*N. arvensis*			*N. unguicularis*			Standard Antibiotic	
	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	A10	NS
B. megaterium	14	12	8	12	10	12	15	14	12	9	11	8	11	ND
B. subtilis	15	13	10	10	11	10	14	11	13	8	13	7	15	ND
B. cereus	12	10	9	9	8	9	12	10	12	9	14	9	12	ND
E. coli	-	11	-	10	8	-	-	7	10	-	12	-	11	ND
K. pneumoniae	10	14	9	10	13	10	10	8	12	8	10	-	17	ND
E. aerogenes	11	13	10	10	14	10	13	12	10	8	10	7	16	ND
S. aureus	12	14	7	9	9	7	12	9	9	9	-	9	16	ND
M. smegmatis	14	13	9	13	13	10	10	13	9	12	9	10	19	ND
M. luteus	10	12	-	10	12	10	11	13	-	9	9	-	33	ND
P. vulgaris	12	11	11	12	12	10	11	10	-	10	12	9	14	ND
L. monocytogenes	10	13	-	10	10	13	9	10	10	11	10	9	12	ND
P. aeruginosa	12	9	9	-	-	-	10	13	10	10	11	-	10	ND
S. cerevisiae	12	11	10	8	8	8	9	7	9	12	15	-	ND	18
C. albicans	15	10	13	10	10	10	10	9	-	14	12	9	ND	18
R. rubra	15	10	13	10	12	-	10	9	-	12	12	8	ND	14
K. fragilis	13	12	10	12	11	8	11	8	9	12	12	8	ND	15

Ch:Chloroform; Ac: Acetone; Me: Methanol; A10: Ampicillin 10 □g; NS: Nystatine 100U;
ND: Not Detected; Control (Acetone, Chloroform and Methanol): No inhibition Zone

Standard Antibiotic	Resistant (mm)	Moderate (mm)	Sensitive (mm)
[1]Ampicillin 10 µg	<11	12-14	>16
[2]Nystatin 30 µg	<12	14-17	>18

Table II. Antimicrobial Activity of Chloroform, Acetone and Methanol Extract of *Nigella* sp. stem

Microorganisms	*N. damascena*			*N. sativa*			*N. arvensis*			*N. unguicularis*			Standard Antibiotic	
	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	A10	NS
B. megaterium	18	8	11	15	-	10	15	13	10	25	10	12	11	ND
B. subtilis	18	8	13	16	7	7	16	13	10	20	10	13	15	ND
B. cereus	19	8	11	16	7	7	15	10	10	28	-	10	12	ND
E. coli	10	-	10	-	8	9	10	8	9	-	9	-	11	ND
K. pneumoniae	12	10	12	8	9	9	10	8	9	22	9	10	17	ND
E. aerogenes	12	9	10	7	8	9	10	8	10	20	9	10	16	ND
S. aureus	10	10	10	7	8	9	11	8	10	28	8	10	16	ND
M. smegmatis	11	11	13	12	9	8	12	8	10	23	8	9	19	ND
M. luteus	11	11	14	10	8	8	12	9	12	25	8	10	33	ND
P.vulgaris	16	8	10	11	-	10	10	-	10	20	8	-	14	ND
L. monocytogenes	15	8	12	10	9	7	12	8	9	-	7	8	12	ND
P. aeruginosa	9	-	12	11	-	7	10	7	-	28	8	-	10	ND
S. cerevisiae	10	-	10	12	9	10	10	10	9	14	9	8	ND	18
C. albicans	10	9	10	12	9	10	10	9	8	17	9	7	ND	18
R. rubra	10	8	9	11	10	11	10	9	-	17	10	-	ND	14
K. fragilis	10	7	10	10	11	10	10	9	8	15	8	-	ND	15

Ch:Chloroform; Ac: Acetone; Me: Methanol; A10: Ampicillin 10 □g; NS: Nystatine 100U;
ND: Not Detected; Control (Acetone, Chloroform and Methanol): No inhibition Zone

Table III. Antimicrobial activity of Chloroform, Acetone and Methanol extract of *Nigella* sp. flowers

Microorganisms	*N. damascena*			*N. sativa*			*N. arvensis*			*N. unguicularis*			Standard Antibiotic	
	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	A10	NS
B. megaterium	15	12	10	14	11	12	17	11	-	16	13	-	11	ND
B. subtilis	15	12	10	14	11	12	15	13	7	16	13	-	15	ND
B. cereus	14	13	10	14	13	12	15	13	7	16	13	-	12	ND
E. coli	10	11	-	10	-	-	10	-	10	12	12	9	11	ND
K. pneumoniae	12	14	-	12	15	10	14	13	9	12	12	12	17	ND
E. aerogenes	10	13	-	12	14	10	16	15	-	12	12	14	16	ND
S. aureus	10	15	9	12	16	10	18	14	12	14	15	12	16	ND
M. smegmatis	16	12	13	10	10	10	14	13	12	15	12	12	19	ND
M.luteus	10	10	11	12	10	10	13	13	10	10	14	7	33	ND
P. vulgaris	14	10	8	10	9	10	16	10	10	10	12	12	14	ND
L. monocytogenes	12	10	8	9	10	10	14	10	13	13	10	10	12	ND
P. aeruginosa	10	8	8	9	10	9	14	8	-	7	10	7	10	ND
S. cerevisiae	13	10	-	12	12	10	10	10	10	9	9	10	ND	18
C. albicans	13	10	-	12	12	10	8	10	-	9	10	10	ND	18
R. rubra	13	10	10	12	10	10	8	12	10	9	10	10	ND	14
K. fragilis	13	10	11	12	12	10	8	12	10	9	12	10	ND	15

Ch:Chloroform; Ac: Acetone; Me: Methanol; A10: Ampicillin 10 □g; NS: Nystatine 100U;
ND: Not Detected; Control (Acetone, Chloroform and Methanol): No inhibition Zone

Table IV. Antimicrobial Activity of Chloroform, Acetone and Methanol extract of *Nigella* sp. capsules

Microorganism	*N. damascena*			*N. sativa*			*N. arvensis*			*N. unguicularis*			Standard Antibiotic	
	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	A10	NS
B. megaterium	15	15	10	15	13	-	21	10	-	12	17	7	11	ND
B. subtilis	15	14	10	15	13	-	20	13	-	12	15	9	15	ND
B. cereus	15	15	10	15	13	12	20	10	-	10	15	9	12	ND
E. coli	14	12	-	9	10	-	-	-	-	-	10	-	11	ND
K. pneumoniae	10	12	10	12	11	11	16	14	10	13	18	-	17	ND
E. aerogenes	10	11	10	14	13	13	11	12	-	13	17	-	16	ND
S. aureus	10	10	11	10	15	10	10	8	10	10	17	10	16	ND
M. smegmatis	10	12	11	17	13	-	10	-	12	13	15	-	19	ND
M. luteus	9	11	8	10	13	9	10	10	10	11	14	-	33	ND
P. vulgaris	10	7	-	10	11	-	-	9	14	10	15	-	14	ND
L. monocytogenes	10	11	-	10	12	9	20	13	10	10	15	10	12	ND
P. aeruginosa	7	7	-	10	10	-	9	8	9	8	10	-	10	ND
S. cerevisiae	9	10	8	10	13	-	10	13	-	12	14	13	ND	18
C. albicans	9	11	9	9	13	7	-	-	-	14	14	8	ND	18
R. rubra	7	10	9	8	12	7	16	12	-	-	12	-	ND	14
K. fragilis	8	10	8	8	15	9	10	10	-	10	12	-	ND	15

Ch:Chloroform; Ac: Acetone; Me: Methanol; A10: Ampicillin 10 □g; NS: Nystatine 100U;
ND: Not Detected; Control (Acetone, Chloroform and Methanol): No inhibition Zone

Table V. Antimicrobial Activity of Chloroforms, Acetone and Methanol extract of *Nigella* sp. seeds

Microorganisms	*N. damascena*			*N. sativa*			*N. arvensis*			*N. unguicularis*			Standard Antibiotic	
	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	Ch	Ac	Me	A10	NS
B. megaterium	8	12	8	12	30	10	-	8	-	-	14	-	11	ND
B. subtilis	8	13	8	15	30	10	-	10	-	11	14	-	15	ND
B. cereus	9	12	8	12	27	10	-	8	-	-	14	-	12	ND
E. coli	9	-	-	-	25	10	-	10	-	-	14	-	11	ND
K. pneumoniae	8	10	8	8	32	10	-	14	8	-	14	-	17	ND
E. aerogenes	8	9	9	8	20	7	7	10	-	-	10	-	16	ND
S. aureus	11	11	8	15	30	10	-	14	-	10	14	9	16	ND
M. smegmatis	11	11	8	12	25	9	-	10	-	-	12	-	19	ND
M. luteus	7	8	-	12	13	12	7	13	7	22	13	-	33	ND
P. vulgaris	8	10	-	10	19	11	-	17	-	10	15	10	14	ND
L. monocytogenes	9	-	-	13	7	7	-	8	-	-	9	-	12	ND
P. aeruginosa	-	12	-	10	13	9	-	-	-	-	10	-	10	ND
S. cerevisiae	-	-	-	9	17	10	9	12	-	-	15	-	ND	18
C. albicans	-	-	-	-	9	10	9	12	-	-	8	-	ND	18
R. rubra	7	-	-	7	12	10	-	9	-	-	15	-	ND	14
K. fragilis	7	-	-	9	15	10	-	10	-	9	15	7	ND	15

Ch:Chloroform; Ac: Acetone; Me: Methanol; A10: Ampicillin 10 □g; NS: Nystatine 100U;
ND: Not Detected; Control (Acetone, Chloroform and Methanol): No inhibition Zone

The extraction product also varied in terms of quality, quantity and composition according to climate, soil composition, plant organ, age etc. (Bakkali *et al*., 2008).

CONCLUSION

This study verified of antimicrobic properties of *Nigella* sp. as a potential antimicrobial agent. *N. sativa* seed extracts possessed the most promising antimicrobial activity against various microorganisms and this could be used as an topical pharmaceutical preparations.

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