ANTIBACTERIAL AND ANTIFUNGAL EFFECTS OF *NIGELLA SATIVA* EXTRACTS AGAINST *S. AUREUS*, *P. AEROGINOSA* AND *C. ALBICANS*

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ABSTRACT

Nigella sativa seeds (blackseed; kalonji) have been used in traditional medicine for the treatment of a variety of diseases including diarrhea and asthma. In this study, the antibacterial and antifungal effects of the aqueous, methanol and chloroform extracts of the seeds against standard and hospital strains of *Candida albicans*, coagulase-positive *Staphylococcus aureus* (*CPSA*) and *Pseudomonas aeroginosa* were investigated and compared with standard drugs, clotrimazole, cloxacillin and gentamicin respectively. Aqueous and methanol extracts were prepared using reflux device and the chloroform extract was prepared by the wetting method. 50 samples from each microorganism were collected from different biological samples such as wound, blood, urine and CSF and the inhibitory effects of the extracts were assessed using agar dilution, cylinder plate and disk diffusion methods. The aqueous extract did not show any effect, but the other extracts showed high inhibitory effects against all the microorganisms in all the three methods and can be suggested as a subject of more extensive investigations in this field.

KEY WORDS: *Nigella sativa*, blackseed, plant extracts, antibacterial activity, antifungal activity

Pak J Med Sci January-March 2005 Vol. 21 No. 1 47-52

INTRODUCTION

Nigella sativa (black cumin; kalonji) is an annual herbaceous plant growing in Western Asia and the Mediterranean region for its seeds. The seeds contain 40% fixed oil, a saponin (melantin) and up to 1.4% volatile oil[1]. The seeds of *Nigella sativa* have been used traditionally for centuries in the Middle East, Northern Africa and South Asia for the treatment of various diseases.[2,3]

The plant extracts and essential oil showed a broad range of pharmacological effects such as antidiabetic[4,5], spasmolytic and bronchodilator[6,7], antioxidant[2,8], hepatoprotective[9,10], antihyperlipidemic[11], analgesic and anti-inflammatory[12], antitumor[13,14] and antiulcer[15,16] effects in various studies. The extracts also showed *in vitro* and *in vivo* antimicrobial[17,18], antileishmanial (unpublished data) and anticestodal effects[19]. It is used traditionally in Iran as laxative, carminative and intestinal antiprotozoal drug[20]. There is increasing problem in microbial resistance and increasing demand for the development of new antimicro-

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* Received for publication: December 8, 2003
1st Revision received: June 2, 2004
2nd Revision received: August 26, 2004
Revision accepted: September 8, 2004

bial agents, especially in hospital settings. As there is no previous study on hospital strains of pathogenic microbes, we decided to study the effects of aqueous, methanol and chloroform extracts of the seeds on some hospital and standard bacterial and fungal strains.

MATERIALS AND METHODS

***Nigella sativa* seeds**: The seeds were collected from herbal drugs shops.

Chemicals: Methanol and chloroform were purchased from Merck Company. Gentamicin was a gift from Darou-Pakhsh pharmaceutical company (Tehran), cloxacillin was a gift from Abou-Reihan pharmaceutical company (Tehran) and clotrimazole was a gift from Jaber-ibn-Hayan pharmaceutical company (Tehran).

Extraction

***Reflux extraction*:** Aqueous and methanol extractions were performed by the following method. 50 g of blackseed powder were used with 300 ml of water or methanol with an extraction period of 10-12 hour. The extracts were filtered using filter paper and the solvents were evaporated using rotary distillation apparatus. In order to obtain a completely dry extract, the resultant extracts were transferred to glass dishes and were left in a 50°C oven for 24 hour. Then, they were left at 4°C until assessment of their antimicrobiological activities.

Wetting procedure: 600 g of ground blackseed in 1500 ml chloroform were incubated in 25°C for one week, during which vibration was carried out up to 5 times a day. The resultant solution was filtered and dried up as previously described in reflux extraction.

Microbiological assessments

Agar dilution method: Known amounts (Table-III) of the extracts were added to 100 ml of Muller-Hinton agar culture media (Merck) to obtain different concentrations in the media. The same procedure was carried out for the antibiotics used. Plates containing 15 ml of these media were incubated in 25°C for 24 hour (for bacteria) and 48 hour (for fungi) to assure that they have not been contaminated during preparation. The microorganisms (standard and hospital strains of *C. albicans*, *CPSA* and *P. aeroginosa*) were cultured on these plates, incubated as mentioned above and the results were recorded for analysis.

Disk diffusion method: The paper disks weighed previously were immersed for half an hour in the solutions of different concentrations of the extracts and were dried out under a laminar flow cabinet. By weighing the dried disks and comparing their weight before immersion, the amount of extracts in disks were calculated. Negative control disks were prepared using the solvent of the extracts in the same way. These (together with positive control disks) have been used for microbiological assay in plates containing the appropriate microorganisms.

***Cylinder plate method*:** After preparing culture media and growing microorganisms, cylinders were placed in plates (5 of them filled with 0.2 ml of different concentrations of the extracts, Table-VI, together with one negative control filled with 0.2 ml of the solvent and 1 positive control filled with 0.2 ml of the standard antibiotics) and incubated at 37°C for 24 hours.

RESULTS

In this study we investigated the antibacterial and antifungal effects of aqueous, methanol and chloroform extracts of *Nigella sativa* seeds on standard and hospital microorganisms *C. albicans*, coagulase positive *S. aureus* (*CPSA*) and *P. aeroginosa*. Table-I shows the number and the location of hospital samples for the microorganisms. As chloroform has bactericidal activity at concentrations of 0.1-0.5%, the antibacterial effects of the chloroform extracts were investigated only by disk diffusion method. The aqueous extract was ineffective in all three methods.

Agar dilution method: As shown in Table-II, the minimum inhibitory concentration (MIC) of the methanol extract were 0.0625 g/100 ml, 0.125 g/100 ml and 1 g/100 ml for hospital samples of *C. albicans*, *S. aureus* and *P. aeroginosa*, respectively. The MICs of the standard drugs against these organisms are shown in Table-III.

Table-I: The location and number of hospital samples of the microorganisms used for microbiological assessment

Coagulase-positive S. aureus			*P. aeroginosa*			*C. albicans*		
Location	*No.*	*Percent*	*Location*	*No.*	*Percent*	*Location*	*No.*	*Percent*
Wound	29	58	Wound	31	62	Wound	3	6
Blood	5	10	Blood	8	16	Vagina	15	30
Urine	2	4	Urine	5	10	Urine	27	54
Ear	1	2	Ear	3	6	Throat	5	10
Eye	3	6	Eye	2	4	-	-	-
Feces	8	16	Throat	1	2	-	-	-
Vagina	1	2	-	-	-	-	-	-
CSF	1	2	-	-	-	-	-	-

Table-II: Inhibitory effects of methanol extract of *Nigella sativa* on hospital microorganisms by agar dilution method

C. albicans			*CPSA*			*P. aeroginosa*		
Conc. g/100 ml	*Growth inhibition (%)*	*Growth (%)*	*Conc. g/100 ml*	*Growth inhibition (%)*	*Growth (%)*	*Conc. g/100 ml*	*Growth inhibition (%)*	*Growth (%)*
0.031	0	100	0.0625	0	100	0.5	0	100
0.0625	62	38	0.125	56	44	1	24	76
0.125	80	20	0.25	78	22	1.5	88	12
0.25	94	6	0.5	96	4	2	96	4
0.5	100	0	1	100	0	-	-	-

Table-III: Inhibitory effects of the standard drugs against hospital microorganisms by agar dilution method

Cloxacillin dose (mcg/ml)	*Coagulase-positive S. aureus*		*Gentamicin dose (mcg/ml)*	*P. aeroginosa*		*Clotrimazole dose (mcg/ml)*	*C. albicans*	
	Growth inhibition (%)	*Growth (%)*		*Growth inhibition* (%)	*Growth (%)*		*Growth inhibition* (%)	*Growth (%)*
2.5	14	86	5	30	70	1	30	70
5	60	40	10	38	62	2	56	44
10	68	32	20	52	48	4	82	18

Disk diffusion method: As shown in Table-IV, the minimum effective concentration of the chloroform extract in the disk for zone of inhibition of more than 20 mm for hospital samples of *C. albicans* was 10mg and of methanol extract was 2mg. The effects of both extracts against the standard samples of the organism were excellent and for the same zone of inhibition were 5mg for chloroform extract and 2mg for the methanol extract.

The minimum effective concentration of the chloroform extract in the disk for zone of inhibition of more than 20 mm for the hospital samples of *CPSA* was 15mg and of methanol extract was 8mg.

The minimum effective concentration of the chloroform extract in the disk for zone of inhibition of 10-20 mm for hospital strains of *P. aeroginosa* was 12mg and of methanol extract was 8mg. The minimum effective concentration of the chloroform extract in the disk for zone of inhibition of 10-20 mm for the standard samples of *P. aeroginosa* was 34mg and of methanol extract was 10mg. Similar results were observed for the extracts against the standard strains of the organisms. The results of the effects of the standard drugs against the hospital samples strains of the microorganisms are shown in Table-V.

Table-IV: The effects of methanol and chloroform extracts of *N. sativa* on hospital samples of microorganisms by disk diffusion method*

Methanol extract in a disk (mg)	*Per cent of microorganism@ with zone of inhibition*			*chloroform extract in a disk (mg)*	*Per cent of microorganism@ with zone of inhibition*		
	> 20 mm	*10-20 mm*	*< 10 mm*		*> 20 mm*	*10-20 mm*	*< 10 mm*
34	-/-/0	-/-/100	-/-/0	34	-/-/0	-/-/38	-/-/62
12	100/100/0	0/0/62	0/0/38	20	88/60/-	12/40/-	0/0/-
8	76/20/0	24/80/6	0/0/94	15	66/22/0	34/70/8	0/8/92
4	24/0/0	76/88/0	0/12/100	10	28/0/0	46/54/2	26/46/98
2	8/0/-	70/54/-	22/46/-	5	0/0/-	20/0/-	80/100/-

* Zone of inhibitions of methanol and chloroform extracts of *N. sativa* against the standard sample of the organisms were similar to the hospital ones.
@ The zone of inhibition numbers shown for *C. albicans*, *CPSA* and *P. aeroginosa* respectively.

Table-V: The antimicrobial effects of cloxacillin, gentamicin and clotrimazole on hospital microorganisms by disk diffusion method*

Antimicrobial agent	*Amount of drug in a disk (mcg)*	*Type of microorganism*	*Per cent of organisms with zone of inhibition*		
			> 20 mm	*10-20 mm*	*< 10 mm*
Cloxacillin	5	*CPSA*	26	14	60
Gentamicin	10	*P. aeroginosa*	30	26	44
Clotrimazole	8	*C. albicans*	82	18	0

* Zone of inhibition for coagulase-positive *S. aureus*, *P. aeroginosa* and *C. albicans* were 25.4, 32.5 and 31.2 respectively.

Table-VI: The effects of methanol extract of *N. sativa* on hospital samples of microorganisms by cylinder plate method*

Extract concentration	*Per cent of microorganisms@ with zone of inhibition*		
mg/ml	*> 20 mm*	*10-20 mm*	*< 10 mm*
170	-/100/16	-/0/84	-/0/0
60	100/42/0	0/58/62	0/0/38
40	92/14/0	8/86/54	0/0/44
20	48/0/0	52/90/20	0/10/80
10	28/-/-	68/-/-	4/-/-

* The extract produced a zone of inhibition of >20 mm in the concentrations of more than 20 mg/ml, 40 mg/ml and 60 mg/ml against standard strain of *C. albicans*, *CPSA* and *P. aeroginosa* respectively.
@ The zone of inhibition numbers shown for *C. Albicans*, *CPSA* and *P. aeroginosa* respectively.

Cylinder plate method: As shown in Table-VI, the MIC of the methanol extract for zone of inhibition of more than 20 mm were 10 mg/ml, 20 mg/ml and 170 mg/ml for the hospital samples of *C. albicans*, *CPSA* and *P. aeroginosa* respectively. The MIC of the methanol extract for zone of inhibition of more than 20 mm were 20 mg/ml, 40 mg/ml and 60 mg/ml for the standard samples (strains) of *C. albicans*, *CPSA* and *P. aeroginosa* respectively.

DISCUSSION

The results showed that the methanol extract of *Nigella sativa* seeds had the best antimicrobial activity and the chloroform extract had a weaker effect. The aqueous extract did not show any antimicrobial activity up to the dose of 2 g/100 ml in agar dilution method and 150 mg/ml in cylinder method. The methanol extract showed the least antimicrobial activity against *P. aeroginosa* and the best activity against *C. albicans*. The results also showed similar effects against both hospital and standard strains of the organisms.

Hanafy and Hatem (1991) also observed antimicrobial activity of diethyl ether extract of the plant in the concentration of 25-400 mcg/disk against *S. aureus*, *P. aeroginosa*, *E. coli* and *C. albicans*. Our results demonstrated effective concentrations of 2-34 mg/disk of methanol extract against the same organisms (except against *E. coli*) that had a significant lower potency than their diethyl ether extract. We used methanol and chloroform extract by disk diffusion method and this may explain the difference between the results. Also, the amount of ingredients of the same plant can be affected by the area and the season of collection. Other aspects of our results are in agreement with Hanafy and Hatem (1991). *S. aureus* and *C. albican* had similar sensitivity against the extracts and were more sensitive than *P. aeroginosa* in both studies. Also, the difference between the higher and lower limits of the range of the concentrations per disk is very similar. They had their higher limit 16 fold of their lower limit while we had it 17 fold. The inhibitory effects of the aqueous extract of the seeds against *C. albicans* have also been shown in mice *in vivo*[18]. We did not find any antimicrobial effect with the aqueous extract. In addition to the possible effects of the factors mentioned above, *in vivo* effect may also been observed as a cooperation of the antimicrobial agent and the immune system.

Nigella sativa seed extracts also showed anticestodal[19] and antileishmania (unpublished data) effects. The mechanism of action of antimicrobial effects of the extracts is not clear but their broad spectrum of activity implies that they should affect basic and common key processes in the organisms.

Finally, our results are in agreement with others who showed that *Nigella sativa* extracts produce antimicrobial activity against a broad range of microbes and especially on multiple antibiotic resistant bacteria[21]. Further studies on the activity-directed fractionation for the isolation of respective pure compounds may result in interesting results.

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