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In vitro antimicrobial activity of ethanolic seeds extract of *Nigella sativa* (Linn) in Sudan

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***Nigella sativa* Lin. (family- Ranunculaceae) is a widely used medicinal plant globally and popular in various Indigenous system of medicines. The seeds are used as astringent, stimulant, diuretics and anthelmintic traditionally. They are also useful for treating jaundice, intermittent fever, dyspepsia, paralysis, piles and skin disorders. The ethanolic extracts of *N. sativa* (seeds) were tested against four standard bacteria, that is, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and against two standard fungi species, that is,. *Aspergillus niger* and *Candida albicans* using the agar plate diffusion method. The ethanolic extracts of *N. sativa* (seeds) exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from 18 to 32 mm in length. The largest inhibition zone in the case of bacteria was obtained for against bacteria *E. coli* (32 mm) while in case of fungi highest inhibition was observed against *Apergillus niger* (25 mm). Thus, it can be concluded from the present study that *N. sativa* possess both antibacterial as well as antifungal activity.**

Key words: *In vitro*, antimicrobial activity, *Nigella sativa* (seeds), Sudan.

INTRODUCTION

Nigella sativa is a herbaceous plant found in the Middle East, Europe as well as the Western and Middle Asia. Its seeds have a great medicinal importance and have been reported to exhibit many pharmacological properties that

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Figure 1. Laboratory sample of *Nigella sativa* seeds.

include antiparasitic, antimicrobial anti-oxidant and anti-inflammatory activities (Ali and Blunden, 2003). Its oil is used as condiment, carminative, food preservative, analgesic and in the treatment of many ailments in different parts of world (Salem, 2005).

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 as mentioned (Brutis and Bucar, 2000; Gilani et al., 2004). As such, *N. sativa* is a natural remedy against many diseases and the aromatic seeds are extensively used as spice, carminative and condiment traditionally, the seeds have been used as adiuretic, diasphoretic, stomachic and liver tonic. As a combination with other ingredients, they are used in diarrhoea (Mansour et al., 2002), indigestion, dyspepsia and sour belching; they also remove foul breath and watering from the mouth, the seeds of *N. sativa* are consumed with buttermilk to cure obstinate hiccups and are also useful in treating loss of appetite, vomiting and dropsy, in different combinations, the seeds have been used in obesity and dyspnoea as well, they have antibilious property and are administered internally in intermittent fevers (Usmanghani et al., 1997). The herbs of *N. sativa* has been regarded as a valuable remedy in hepatic and digestive disorders, constant inhalation of fried seeds relieves cold and catarrh. They have also been used in chronic headache and migraine (Evans, 1996). The decoction of seeds with some sweet oil forms a useful application or ointment in skin diseases, the seeds have been useful in mercury poisoning, sores and leprosy (Evans, 1996). Brayed in water, its application removes swelling from hands and feet, *N. sativa* is also used externally in leucoderma, alopecia, eczema, freckles and pimples (Usmanghani et al., 1997). The purpose of the present study was to evaluate the antimicrobial activity of *N. sativa* against few common bacterial and fungal species.

MATERIALS AND METHODS

The *N. sativa* seeds were collected from central Sudan (Khartoum) between January and February 2008. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) (Figure 1). The *N. sativa* seeds were air-dried, in shade with good ventilation and then ground finely in a ml until their use for extracts preparation.

Preparation of crude extracts

Extraction was carried out from the seeds of *N. sativa* (Seeds) plant by using overnight maceration techniques (Harborne, 1984). About 50 g of round seeds material was macerated in 250 ml of ethanol for 3 h at room temperature. With occasional shaking for 24 h at room temperature, the supernatant was decanted and filtered through a filter paper. After filtration, the solvent was then removed under reduced pressure by rotary evaporator at 55°C. Each residue was weighed and the yield percentage was calculated and then stored at 4°C in tightly sealed glass vial ready for further use. The remaining extract which was not soluble was successively extracted by ethanol. Using the previous technique, extracts were kept in deep freezer for 48 h, and then induced in freeze dryer (Virtis, USA) until completely dried. The residue was weighed and the yield percentage was calculated. The extracts were kept in 4°C until the time of their use.

Test microorganisms

The ethanolic extract of *N. sativa* seeds were tested against four bacterial species: two Gram-positive bacteria viz., *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Also, the same was tested against two fungal strains viz, *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596). The bacterial and fungal strains used in the study were obtained from the Department of Microbiology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) and National Health Laboratory of Khartoum in Sudan.

The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used in the antimicrobial test.

Table 1. The antimicrobial activity of *N. sativa* seeds against the standard bacterial and fungal.

Standard microorganisms	Concentration (mg/ml)			
	Mean diameter of growth inhibition zone (mm)			
	100	50	25	12.5
Tested bacteria used (M.D.I.Z. mm)				
Bacillus subtilis	20	20	19	18
Staphyococcus aureus	18	17	16	16
Escherichia coli	32	25	22	20
Pseudomonas aeruginosa	20	19	18	16
Tested fungi used (M.D.I.Z. mm)				
Apergillus niger	25	22	20	19
Candida albicans	21	20	19	18

Interpretation of results: MDIZ (mm) : >18 mm: sensitive, 14 to 18 mm: intermediate: <14 mm: resistant. (-): no inhibition.

In vitro testing of extracts for antimicrobial activity

Antibacterial testing: The cup-plate agar diffusion method as reported (Kavanagh, 1972) was used adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. 1 ml of the standardized bacterial stock suspension (between 10^8 and 10^9 CFU/ml) was thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dish plates. The agar was left to set and in all of these plates, 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Each cup was filled with 0.1 ml sample of the ethanolic extracts using an automatic microlitre pipette, and thereafter the extracts were allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 18 h. Two replicates were carried out for each extract against each of the test organisms. After incubation, the diameters of the resultant growth inhibition zones were measured and averaged. The mean values were tabulated.

Antifungal testing: The same method used for the antibacterial test was employed. However, the growth media used in case of fungi, was Sabouraud dextrose agar instead of nutrient agar. The inoculated medium was incubated at 25°C for two days for *C. albicans* and three days for *A. niger*.

RESULTS AND DISCUSSION

The seeds of *N. sativa* family (Ranunculaceae) were screened for antimicrobial activity against two Gram positive bacteria (*B. subtilis*, *S. aureus*), two gram negative bacteria (*E. coli*, *P. aeruginosa*) as well as two fungi (*A. niger* and *C. albicans*) using the cup plate agar diffusion method screened.

The extracts obtained from the seeds of *N. sativa* exerted a pronounced activity against all bacteria and fungi strains tested. This was indicated by diameter of growth inhibition zones that varied from 18 to 32 mm. This result was similar to an earlier report (Khalid et al., 2011). These authors found that methanolic and aqueous extracts of the seeds of *N. sativa* possessed antibacterial activity against *B. subtilis, Entereococcus faecalis, P. aeruginosa, S. aureus* and *Salmonella typhi*.

Previous study on *N. sativa* crude plant extracts and phytoconstituents also supports the fact that *N. sativa* is active against various pathogens. In the past, many researchers investigated the antimicrobial potential of *N. sativa*. They found that *N. sativa* ethanolic extracts was active against a tested standard microorganism and multidrug resistant strains of bacteria (Salman et al., 2005). Our results were in agreement with those reported by Ani et al. (2006), which indicated that *N. sativa* has a significant antibacterial potential against *B. subtilis* and *B. cereus*. Thus, *N. sativa* with an array of polyphenolic compounds, possess antibacterial activity (Ani et al., 2006). In 1975, the purified compound thymohydroquinone (THQ) from *N. sativa* oil (NSO) was found to possess a high antimicrobial effect against Gram positive microorganisms (El-Fatatry, 1975). In later studies, the seed extracts of *N. sativa* were found to inhibit the growth of *E. coli*, *B. subtilis* and *Streptococcus feacalis* (Saxena and Vyas, 1986).

The antimicrobial activity of *N. sativa* was further established against several species of pathogenic bacteria and yeast (Topozada et al., 1965; Hanafy and Hatem, 1991). The result of minimum inhibition concentration from Table 1 shows that 12.5 µg/ml was the lowest concentration at which all the tested micro-organisms were inhibited. A comparison of observation given in Tables 2 and 3, show that the seed extracts of *N. sativa* dissolved in dimethyl sulphoxide inhibited all bacteria higher than 40 µg/ml ampicillin and a lower concentration of gentamicin. The seed extracts of *N. sativa* inhibited *E. coli* at 40 µg/ml, which was similar to the result obtained for gentamicin. The seeds extracts of *N. sativa* inhibited *A. niger* with a higher than 20 µg/ml of Clotrimazole, and inhibited *C. albicans* at more than 50 µg/ml of Nystatin. It is clear from Table 1 that the ethanolic extract of *N. sativa*

Table 2. Antimicrobial activity of *N. sativa* seeds against the standard bacterial and fungal species.

Standard microorganisms	Mean diameter of growth inhibition zone (mm)
Tested bacteria used (M.D.I.Z. mm)	
Bacillus subtilis	20
Staphyococcus aureus	18
Escherichia coli	32
Pseudomonas aerugino	20
Tested fungi used (M.D.I.Z. mm)	
Apergillus niger	25
Candida albicans	21

Interpretation of results: MDIZ (mm) : >18 mm: sensitive, 14 to 18 mm: intermediate: <14 mm: resistant. (-): no inhibition.

Table 3. Antibacterial and antifungal activity of reference antibiotics against standard microorganisms.

Drugs	Concentrations (µg/ml)	Standard microorganisms used MDIZ* (mm)			
		Gram (+ve)		Gram (-ve)	
		Tested bacteria used (M.D.I.Z. mm)			
		Bacillus subtilis	***Staphyococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
Ampicillin	40	15	25	-	16
	20	14	20	-	13
	10	13	18	-	12
	5	12	15	-	-
Gentamicin	40	29	35	32	23
	20	22	33	30	22
	10	20	30	17	21
	5	17	28	-	19
Tested fungi used (M.D.I.Z. mm)					
		Aspergillus niger		***Candida albicans***	
Clotrimazole	40	30		42	
	20	22		40	
	10	19		33	
	5	16		30	
Nystatin	50	28		17	
	25	26		14	
	12.5	23		-	

MDIZ (mm) = Mean diameter of growth inhibition zone in mm. Interpretation of results: MDIZ (mm); >18 mm: sensitive, 14 to 18 mm: intermediate, <14 mm: resistant. (-): no inhibition.

seeds show a high activity against all bacteria and fungi.

Conclusion

Ethanolic seeds extract of *N. sativa* produced antimicrobial activity against all organisms tested. This study observes that *N. sativa* has useful antimicrobial properties. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

Conflict of interests

The authors did not declare any conflict of interest.

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