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Antimicrobial and antioxidant activity of *Nigella sativa* Linn seeds

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ABSTRACT

In-vitro antimicrobial and antioxidant activities of various concentrations of alcoholic extracts of *Nigella sativa* seeds were examined on four bacterial strains (*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium*) fungal strains used were *Acremonium strictum, Penicillium olivicolor, Aspergillus tereus , Aspergillus versicolor, Aspergillus niger and Aspergillus wentii* gr. Agar disk diffusion method was used to evaluate antimicrobial activity and DPPH (1,1-diphenyl-2-picrylhydrazyl) assay and Riboflavin Photo-oxidation assay for antioxidant activity. The extracts showed strong sensitivity to all the organisms. The zone of inhibition was found maximum in petroleum ether extract of 30 mm at a dose of 250µg/ml against *Salmonella typhimurium* and in fungal strains methanolic extract had a good effect on *Acremonium strictum* with an inhibition zone of 24 mm at a dose of 500µg/ml. The antioxidant activity showed that the extracts exhibited scavenging effect in concentration-dependent manner. The results of DPPH and Riboflavin oxidation method, it was found that compound displayed strong antioxidant activity of 71.22% and 85.49% respectively as compared to ascorbic acid.

KEY WORDS: ANTIMICROBIAL ACTIVITY, ANTIOXIDANT ACTIVITY, NIGELLA SATIVA, DPPH

INTRODUCTION

Nigella sativa is an economically important umbel growing wild in the dry temperature regions belonging to the botanical family of Ranunculaceae is an annual flowering plant, native to south and southwest Asia. It was first identified and described by Linnaeus in 1753 (Jansen *et al.*, 1981). The plant is an erect profusely branched herb that can attain heights of 40-70cm. It bears alternate leaves, terminal white flowers and capsule like fruits. The flowers are delicate, and usually colored pale blue and white, with five to ten petals. The fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous seeds. The latter are filled with black ovoid or obpyramidal seeds attaining lengths and widths ranging from 2.5 to 3.5mm and widths from 1.5 to 2mm. respectively. The plant is known to all Arabian and Islamic countries and carries various colloquial names. It is known generally by the names Habbat Albarakah, Alhabahat Alsawda and Alkamoun Alaswad (Saad *et al.*, 2009).

Of all the plant organs it is only the seeds which have attracted most of the researchers starting from Egypt and the Sudan in Africa and extending to Saudi Arabia, India and Pakistan in Asia and most recently those in Japan, France, England, Canada and USA. Studies conducted over past 2 decades have revealed a multi-range of actions that covered almost all known ailments of mans various body systems. Traditionally, it is used as a natural remedy for a number of illnesses that include asthma, cough, hypertension, bronchitis,

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diabetes, headache, eczema, fever, inflammations, and other diseases (Morsi, 2000; Saad *et al*., 2009).

This seed is commonly used in recipes in Asian countries and it is reported that it possess several medicinal properties (Ali and Blunden, 2003; Randhawa *et al*., 2002). Crude extract and essential oil possess antibacterial activity against several bacteria (Ali and Ghamdi, 2001; Mouhajir and Pedersen,1999; Khan, 2013). Previous work on *Nigella sativa* seeds has shown about its various activities. A few antimicrobial work of the volatile oil of the seeds of

Nigella sativa has been reported (Toppozada *et al*., 1965; Taha *et al*., 1975; Agarwal *et al*., 1979; Hanafy *et al*., 1991; Hasan *et al*., 1989; Gyamfı and Aniya, 2002). Antibacterial activity of honey alone and in combination with *Nigella sativa* seeds against *Pseudomonas aeruginosa* infection has proved quite fruitful (Abdelmalek *et al*., 2013).

The effect of *N. sativa* is most likely due to its protection against cellular damage caused by oxidative stress. The anti-oxidant properties of black seed oil have been reviewed by Ali and Blunden (2003). Various investiga-tors have revealed the antioxidant activity of *N. sativa* (Nagi *et al*., 1999; Turkdogan, *et al*., 2000; Mahmoud *et al*., 2000). Reactive oxygen species (ROS) is one of the areas that is a focus of great interest to researchers in life sciences. The present work is aimed for testing the free radical scavenging activity by using DPPH and Riboflavin oxidation method.

MATERIALS AND METHODS

PLANT MATERIAL

Nigella sativa seeds were collected locally from Srinagar and identified at Department of Botany, University of Kashmir, Srinagar.

EXTRACTION OF PLANT MATERIAL

15 gram powdered sample of *Nigella sativa* seeds was extracted with Petroleum ether and Methanol and Aqueous by cold extraction method. The extracts were filtered through Whatman.No.1 filter paper, evaporated on water bath and stored at 4C for further use.

TEST ORGANISMS

The test microorganisms used in this study (bacteria: *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium*; fungi: *Acremonium strictum, Penicillium olivicolor, Aspergillus tereus, Aspergillus versicolor, Aspergillus niger* and *Aspergillus wentii* gr) were obtained from Bacteriological and Mycological section of Department of Microbiology, SKIMS, Soura, Srinagar.

ANTIMICROBIAL ACTIVITY

The *in vitro* antibacterial activity test was carried out using the disk diffusion method (Bauer *et al*., 1966) and Well diffusion method . The zone of inhibition of extracts and standard control drug were calculated against the test strains.

ANTI-OXIDANT ACTIVITY ASSAYS

For evaluation of anti-oxidant activity of three extracts of *Nigella sativa* following two methods were followed:

DPPH ASSAY

The anti-oxidant activity of both the extracts of the plant was measured with 1, 1-diphenyl 2-picryl hydrazyl radical (DPPH) spectrophotometrically at 517 nm (Blois, 1958). The stock solution of both the plant extracts (5mg/ml) was prepared by dissolving a known amount of dry extract in 10% Aqueous DMSO. The working solutions (50, 100, 150, 200, 250 and 300µg/ml) of all the extracts were prepared from the stock solution using suitable dilution. The scavenging activity was observed by bleaching of DPPH solution from violet colour to light yellow and Ascorbic acid was used as control.

SUPEROXIDE ANION RADICAL SCAVENGING ACTIVITY-RIBOFLAVIN PHOTO-OXIDATION METHOD

Measurement of superoxide anion scavenging activity of both the extracts of the plant was calculated in accordance to the method (Liu *et al*., 1997) spectrophotometrically at 590nm using Phosphate buffer (also taken as control) as Blank after illumination for 5 minutes.

The percentage of inhibition of the free radicals in the above mentioned methods was calculated by using the formula:

$$\% \text{ Inhibition} = \frac{\text{Ac-As}}{\text{Ac}} \times 100$$

Where 'Ac' is the absorbance of the blank and 'As' is absorbance of sample.

RESULTS

ANTIMICROBIAL ACTIVITY

The antimicrobial activities of different concentrations ranging from 125g/ml to 250µg/ml and 100µg/ml to 500µg/ml of three extracts of Nigella sativa (table 1 and 2) were determined against different bacterial and fungal strains respectively and recorded as inhibition zone diameter (IZD), measured in 'mm' methanol as negative control, chloramphenicol as positive control for bacteria and fulconazole for fungi. The results of the antimicrobial activity of the investigated extract are shown in Tables 1 and 2. In this study, petroleum ether, methanol and aqueous extracts showed no inhibition against all the bacteria tested at lower concentrations (125 µg/ml). Generally, the petroleum ether and methanol extract of *Nigella sativa*

exhibited higher antibacterial effect compared with aqueous extracts. The petroleum ether showed inhibitory effects against all the tested bacterial strains except *Klebsiella pneumoniae* with highest inhibition zone diameter of 30mm for *Salmonella typhimurium* at 250µg/ml concentration and the lowest 20mm against P. aeruginosa at 250µg/ml concentration. The inhibition zone of the tested strains at different concentrations ranged between 20-30mm. Methanol extract of the plant showed highest inhibitory activity against *K. pneumonia* and Salmonella typhimurium 20mm at 250µg/ml concentrations and no effect was seen on *Escherichia coli*. However, aqueous extract of the seeds showed highest inhibitory activity against *Klebsiella pneumoniae* 28mm at 250µg/ml whereas *Salmonella typhimurium* showed resistance. The higher concentration methanol extract exhibited antifungal activity against Acremonium strictum strains with maximum inhibition zone of 24mm for *C. albicans* followed by petroleum ether with the inhibition zone of 14mm for *Penicillium olivicolor*. The results showed the activity of three extracts was purely dose dependent with lower concentrations showing no activity and higher concentrations showing maximum activity.

ANTIOXIDANT ACTIVITY

The antioxidant activity of both the extracts as measured by the ability to scavenge DPPH free radicals was compared with the standards/ ascorbic acid. The results (table 3) for the DPPH assay revealed that both the three extracts exhibited significant antioxidant activity. The highest %age inhibition was shown by petroleum ether extract with a maximum of 71.22% inhibition compared to the positive control (Ascorbic acid 97%) at 300µg/ml followed by methanol (50.25%). While as the highest %age inhibition was ones again exhibited by petroleum ether extract (85.42%) at 300µg/ml concentration followed by aqueous (77.71%) as determined by Riboflavin photo-oxidation method.

DISCUSSION

The antimicrobial activity of the extracts of *Nigella sativa* was evaluated against the different clinical strains of bacteria (*E. coli, P. aeruginosa, K. pneumoniae, S. typhimurium*) and fungi (*Aspergillus versicolor, Aspergillus tereu, Penicillium olivicolor, Aspergillus niger, Acremonium strictum and Aspergillus wentii* gr) supported the scientific validity of the plant seeds being used traditionally as a medicine. *N. sativa* seed extracts have been found to possess remarkable antibacterial activity. In the present study three different extracts (petroleum ether, methanol and aqueous) of *N. sativa* showed pronounced activity against *Klebsiella pneumoniae* and *Escherichia coli* and low activity against *Salmonella typhimurium*. Similarly, in a study conducted on the extract of *N. sativa* it was found to be effective on Gram negative bacteria. The Gram positive bacterial strains were resistant due to the fact that they possess an outer membrane which acts as a barrier to many environmental substances including antibiotics (Jigna *et al*., 2007). Antimicrobial activity of different extracts may also be attributed to the presence of soluble phenolic and polyphenolic compounds (Kowalski *et al*., 2007). The results are also in confirmation with a recent study (Bandh *et al*., 2011) in which it was shown that the methanol extract of *Nepeta cataria* inhibited the growth of all the bacterial and fungal test organisms, with maximum inhibitory effects on *S. aureus, P. multocida* and *E. coli* and a minimum effect on *A. flavus*. Thus suggesting that the antimicrobial activity of the extract may be related to the monoterpenoid component i.e., nepeta-lactone. The lack of antibacterial activity in some of the concentrations of the extract is not surprising as a number of plant extracts have been found ineffective against certain test organisms at lower concentrations and may be attributed to the presence of lesser amounts of the antimicrobial compounds.

The antibacterial effects of the extracts could be explained by disturbance of the permeability barrier of the bacterial membrane structure (Cowan *et al*., 1999). The results of the antimicrobial activity of the investigated extract are shown in which the petroleum ether, methanol and aqueous extracts showed no inhibition against all the bacteria tested at lower concentrations (125 µg/ml). Generally, the petroleum ether and methanol extract of *Nigella sativa* exhibited higher antibacterial effect compared with aqueous extracts as suggested by (Hasan *et al*., 2013), in which the two methanol and aqueous extracts were tested against bacterial and fungal strain and it was found that both methanol and aqueous extracts showed no inhibition against all the bacteria tested at lower concentrations (<50 mg/mL). Generally, the methanol extract of *Nigella sativa* exhibited higher antibacterial effect compared with aqueous extracts.

Another recent study conducted by Shahid *et al*., (2013) on antibacterial activity *in vitro* of medicinal plants which revealed that the plant extracts inhibited bacterial growth but their effectiveness varied. Ethyl acetate extract of selected plants showed higher inhibition against tested bacteria at high concentration. While methanol and ethanol extract of selected plants had more significant effect on various tested bacteria as compared to ethyl acetate extract. The antibacterial activity has been attributed to the presence of some active constituents in the extracts. The demonstration of broad spectrum of antimicrobial activities by the plants used in this study may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of gastrointestinal, wound infections and typhoid fever.

Similarly (Abdelmalek et al., 2013) studied the antibacterial activity of honey alone and in combination with *Nigella sativa* seeds against *Pseudomonas aeruginosa* infection which proved fruitful. in which in vitro activities, of three honeys sample, and *Nigella sativa* against *Pseudomonas aeruginosa* alone and in combination and an additive effect between honey and *N. sativa* as regards of their three varieties of honeys studied against *P. aeruginosa* showed that the MIC for the three varieties of honeys were by decreasing order of effect.; 3% (v/v) and 12% (w/v), 2% (v/v) and 14% (w/v), and 2% (v/v) and 10% (w/v).

The extract from the seeds of *Nigella sativa* was screened for antifungal activities against seven strains. The activities were compared with standard Fluconazole. It was noted that

the low concentration 100µg/ml of all the three extracts did not show any activity against six strains. The petroleum ether extract showed moderate inhibition against *Acremonium strictum, Penicillium olivicolor* and *Aspergillus niger* and no activity in rest of the three strains.

The methanolic extract concentration 500µg/ml showed very strong inhibition (24 mm) against *Acremonium strictum* and it showed low inhibition in rest of the fungal strains. While as the aqueous extract showed no significant inhibition in any of the strains. This is in consonance with the results of Shale *et al.* (1999) reporting that water is less effective than other alcoholic solvents at extracting the active compounds from plants. In the present study it was observed that the extracts possessed a dose dependant anti-bacterial and anti-fungal activity. Some researchers attributed the antifungal activity to the phenolic compounds. The amphipathicity of these compounds can explain their interactions with bio-membranes causing the inhibitory effect (Veldhuizen *et al.*, 2004). Previous reports also showed concentration-dependent inhibition of both Gram-positive and Gram-negative bacteria (Hanafi and Hatem, 1991). Thus, extracts showed comparable activity against the bacterial and fungal strains at relatively higher concentration of extracts (250µg/ml for bacteria and 500µg/ml for the fungal strain).

Reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, ironoxygen complexes, hydrogen peroxide and lipid peroxides are generated by several oxidative reactions. Although ROS can help the immune system to clear out extrusive microorganisms, excessive ROS can also react with biological molecules such as DNA, proteins and phospholipids, and eventually cause oxidative damage in tissues and free radical related diseases such as inflammation, heart disease, diabetes, gout, cancer, etc. For aerobic organisms, the major system of defense against oxidative damage is the use of 'antioxidants' to convert excessive ROS into non toxic compounds. An imbalance between the amount of ROS and antioxidant enzymes is a problem for our health. This is why the daily intake of foods with antioxidant activity is necessary (Lee *et al.*, 2005).

The property of plant extracts to scavenge these ROS has been evaluated using separate assays for different type of Reactive oxygen species. 1, 1-Diphenyl 2-Picryl Hydrazyl (DPPH) is a relatively stable radical. Its assay is based on the measurement of the scavenging ability of antioxidants towards DPPH, a nitrogen-centered radical, which reacts with suitable reducing agents. The electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up (Blois, 1958).

The DPPH free radical scavenging activity is due to the neutralization of DPPH free radical by the plant extract, either by transfer of hydrogen or of an electron (Shimada *et al.*, 1992). The DPPH radical has been used widely to test the potential of the compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. Due to strong DPPH scavenging property of Ascorbic acid it is used as a standard antioxidant. The results show that petroleum ether extract of *Nigella sativa* may have hydrogen donors thus scavenging the free radical DPPH, with highest scavenging activity (71.22%) than rest of the plant extracts and the standard antioxidant ascorbic acid with 97% inhibition, recorded at higher extract concentration (300µg/ml). The extracts of this plant scavenged free radicals in a dose-dependent manner corresponding with the results of the extracts of C. fistula (Siddhuraju *et al.*, 2002).

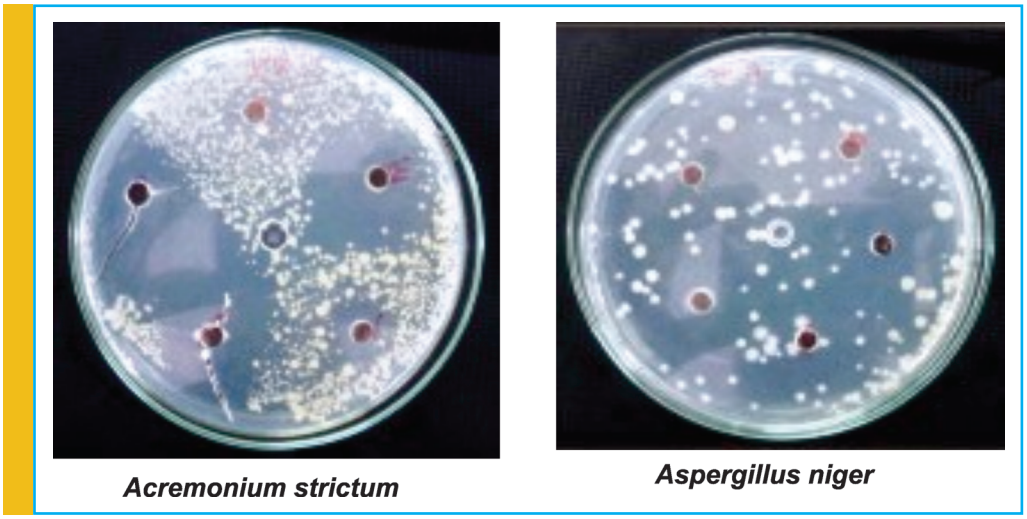
Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl

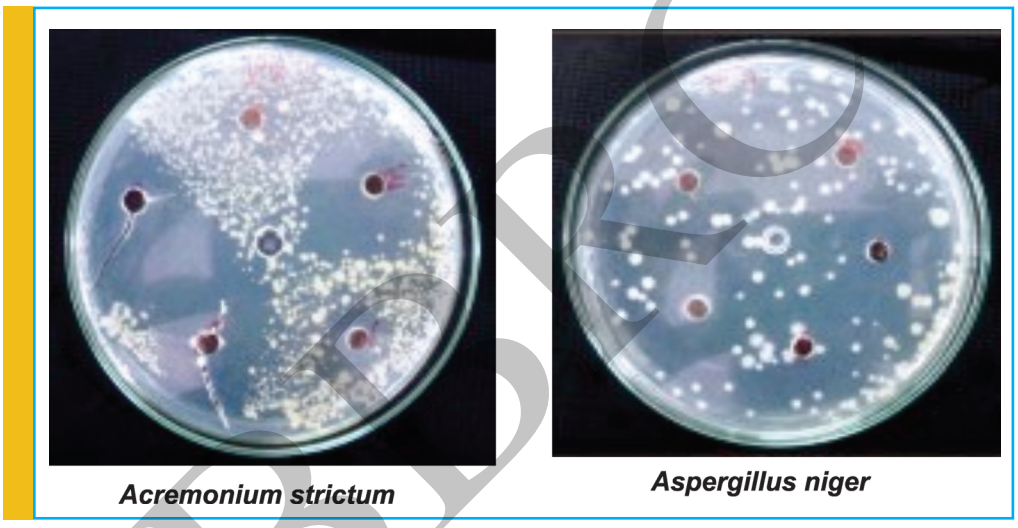
TABLE 1: Antimicrobial activity of three extracts of *N. sativa L.*

Fungal Strains	Inhibition zone diameter (IZD) mm					
	Petroleum ether		Methanol		Aqueous	Chloramphenicol
	125 µg/ml	250 µg/ml	125 µg/ml	250 µg/ml	125 µg/ml	250 µg/ml
Pseudomonas aeruginosa	-	20	-	10	-	14
Salmonella typhimurium	-	30	-	20	-	-
Klebsiella pneumoniae	-	-	-	20	-	28
Escherichia coli	-	26	-	-	-	19

TABLE 2: Antifungal activity of three extracts of *Nigella sativa L.*

Fungal Strains	Inhibition zone diameter (IZD) mm						
	Petroleum ether		Methanol		Aqueous		Fluconazole
	100 µg/ml	500 µg/ml	100 µg/ml	500 µg/ml	100 µg/ml	500 µg/ml	
Acremonium strictum	-	12	-	24	-	-	28
Penicillium olivicolor	-	12	-	10	-	8	28
Aspergillus tereus	-	8	-	-	-	-	10
Aspergillus versicolor	-	-	-	10	-	8	22
Aspergillus niger	-	14	-	6	-	2	18
Aspergillus wentii gr	-	8	-	6	-	4	10





radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA. It is also reported that the products of lipid peroxidation, such as malonyldialdehyde (MDA), damage the enzyme systems and even the DNA. Therefore, studying the scavenging activity of plant extracts on superoxide radical is one of the most important ways of clarifying the mechanism of antioxidant activity (Przybyszewski *et al*., 2005). The results show that petroleum ether of *N. sativa* exhibited good superoxide anion radical scavenging activity (85%) at highest extract concentration (300µg/ml).

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