*Asian Journal of Chemistry* Vol. 21, No. 1 (2009), 467-470

# Antimicrobial Activity of Black Cumin Seeds (*Nigella sativa* L.)

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This study was designed to examine the *in vitro* antimicrobial activity of *Nigella sativa* seed by the paper disc agar diffusion method. For this purpose, extracts of *Nigella sativa* seeds were prepared in ethyl acetate, methanol, acetone, hexane, diethyl ether, chloroform, ethanol tested on bacterial strains of *Staphylococcus aureus* ATTC 6538, *Listeria monocytogenes* SCOTT A, *Bacillus megaterium* NRS, *Micrococcus luteus* LA 2971, *Bacillus subtilis* ATTC 6633, *Yersinia enterocolitica* O:3 P 41797, *Bacillus brevis* EMC 33, *Escherichia coli* ATTC 8739, *Bacillus megaterium* DSM 32, *Pseudomonas aeroginosa* ATTC 9027, *Klebsiella pneumoniae* ATCC 13883, *Enterococcus faecalis* ATCC 15753, *Salmonella typhimurium* ATCC 13311, *Serratia* spp. (isolated in KSU) and *Candida albicans* ATTC 10194 as *in vitro*. The results were compared with ampicillin (10 mcg) and nystatin (30 mcg) standards. It was found that inhibitory activity of *Nigella sativa* seeds showed different levels of antimicrobial activity to all test strains.

**Key Words: *Nigella sativa*, Antimicrobial activity.**

## INTRODUCTION

Various medical plants have been used for years in daily life to treat disease all over the world. Turkey is an important floristic center internationally because of its geographic location, climate and the presence of nearly 10,000 natural plant species. According to a study[1] performed by the WHO based on publications on pharmacopoeias and medical plants in 91 countries, the number of medicinal plants is nearly 20,000. The characteristics of the plants that inhibit microorganisms and are important for human health have also been researched[2-7].

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Nigella (*Nigella sativa* L.) is an annual herbaceous plant belonging to the family Ranunculacea. It is grown in Mediterranean region of Turkey and Cyprus[3]. The seeds are known as black cumin. For thousands of years, the seeds have been used for medical purpose. The seeds are used as seasoning for vegetables, legumes and different types of baked products[4]. It has been used as a herbal medicine for more than 2000 years. It is also used as a food additive and flavour in many countries. *N. sativa* volatile oil has recently been showed to contain 67 constituents, many of which are capable of inducing beneficial pharmacological effects in humans[5]. In several studies, the volatile oil has been shown to have insecticide, bronchodilator, immunomodulative[6], antibacterial[7], hypotensive[8], choleretic, antitumoral[9], antifungal, antihelmentic and antiastmatic[10]. In Turkish folk medicine Nigella seeds are used as a natural stimulant of immunity, antiallergic, asthma, treatment of neurologic and skin diseases, cough, antiinflammatory, analgesic, diuretic, antidiabetic, digestive disorders, carminative, anthelmintic and appetitive[11]. *N. sativa* seeds contain a variable amount of oil, with linolenic generally recognized as the more abundant fatty acid; relevant amounts of saturated acids such as palmitic, myristic and stearic were found in some cases, as well as the presence of unusual unsaturated c20 (PUFA) acids[12].

The aim of this study was to compare the antimicrobial properties of ethyl acetate, methanol, acetone, hexane, diethyl ether, chloroform and ethanol extracts of the *Nigella sativum* seeds on different bacterial strains and fungi or not and to make a source for detailed studies which will be made in future.

## EXPERIMENTAL

The tested microorganisms in this study were provided from the culture collections of the Microbiology Laboratory of the Science and Art Faculty, University of Kahramanmaras Sütçü Imam, Kahramanmaras, Turkey. *Staphylococcus aureus* ATTC 6538, *Listeria monocytogenes* SCOTT A, *Bacillus megaterium* NRS, *Micrococcus luteus* LA 2971, *Bacillus subtilis* ATTC 6633, *Yersinia enterocolitica* O:3 P 41797, *Bacillus brevis* EMC 33, *Escherichia coli* ATTC 8739, *Bacillus megaterium* DSM 32, *Pseudomonas aeroginosa* ATTC 9027, *Klebsiella pneumoniae* ATCC 13883, *Enterococcus faecalis* ATCC 15753, *Salmonella typhimurium* ATCC 13311, *Serratia* spp. (isolated in KSU) bacteria and *Candida albicans* ATTC 10194 fungus were used.

Discs injected with pure diethyl ether, chloroform, ethanol, ethyl acetate, methanol, hexane and acetone served as negative controls. Standard antibiotic discs such as ampicillin (10 mcg) and nystatin (30 mcg) used for comparison and also used as positive controls.

Seeds of *Nigella sativa* were purchased from the herbal markets in Kahramanmaras, Turkey. The seeds were botanically authenticated by a specialist of plant taxonomy in Biology Department. The seeds were ground in a breaker until they would pass a 1 mm sifter and they were preserved in cloth bags in the laboratory until extraction procedure.

**Preparation of model extracts:** The plants seeds were broken into small pieces under sterile conditions and 10 g of each *Nigella sativa* seeds were extracted with 150 mL of diethyl ether, chloroform, ethanol, ethyl acetate, methanol, hexane and acetone solvents (Merck) for 72 h by using Soxhlet equipment[13].

**Test of antimicrobial activity:** In present study, the disc difusion technique was applied. All the extracts thus obtained were injected into empty sterilized antibiotic disks of 6 mm diameter in amounts of 20 µL. All the bacteria mentioned above were incubated at 37 ± 0.1 °C for 24 h by inoculation into nutrient broth. Sterilized petri dishes (9 cm diameter) were inoculated with 0.01 mL of one of the above culture media (105-106 bacteria per mL). Muller-Hinton agar (Merck) sterilized in a flask and cooled to 45-50 °C was distributed by pipette (15 mL) into each inoculated petri dish and swirled to distribute the medium homogeneously. Disks injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri dishes were placed at 4 °C for 1-2 h and then incubated at 37 ± 0.1 °C for 16-24 h. At the end, the inhibition zones formed on the media were measured[13,14]. Antibacterial activity studies were carried out duplicate for each test strains and average measurement were calculated.

## RESULTS AND DISCUSSION

Results of the antimicrobial activity of *Nigella sativa* seeds were shown in Table-1. In this study, 20 µL/disc concentration of extracts of *Nigella sativa* seeds were prepared in ethyl acetate, methanol, acetone, hexane, diethyl ether, chloroform and ethanol tested on 14 bacteria strains and 1 fungus with disc diffusion method as *in vitro*.

It is determined that 20 µL/disc *Nigella sativa* seeds showed antimicrobial effects on all tested bacteria and fungus with inhibition zones between 7-11 mm. *S. aureus* ATTC 6538 was found to be more sensitive strain among bacteria and fungus. On the other hand *S. typhimurium* ATCC 13311 and *B. megaterium* DSM 32 were found to be most resistant bacteria against the *Nigella sativa* seeds extracts. In conclusion, the present results indicated that the extracts of *Nigella sativa* seeds which were prepared using ethyl acetate, has inhibitory activity on almost all microorganisms strains (Table-1).

TABLE -1
RESULTS OF ANTIBACTERIAL ACTIVITY OF *Nigella sativa* SEEDS

| Microorganism | Diameter of inhibition zone (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Extracts (20 µL/disc) | | | | | | | Standards | | Con. |
| | a | b | c | d | e | f | g | Amp | Nst | a,b,c, d,e,f,g |
| *Staphylococcus aureus* ATTC 6538 | 8 | 8 | 9 | 11 | 8 | 9 | 9 | 12 | NT | 0 |
| *Listeria monocytogenes* SCOTT A | 8 | 7 | 0 | 0 | 7 | 8 | 8 | 12 | NT | 0 |
| *Bacillus megaterium* NRS | 7 | 0 | 0 | 7 | 7 | 7 | 0 | 20 | NT | 0 |
| *Micrococcus luteus* LA 2971 | 7 | 7 | 8 | 0 | 7 | 0 | 8 | 10 | NT | 0 |
| *Bacillus subtilis* ATTC 6633 | 7 | 7 | 9 | 0 | 7 | 0 | 8 | 17 | NT | 0 |
| *Yersinia enterocolitica* O:3 P 41797 | 7 | 8 | 0 | 0 | 0 | 0 | 0 | 12 | NT | 0 |
| *Bacillus brevis* EMC 33 | 8 | 7 | 0 | 7 | 0 | 7 | 8 | 17 | NT | 0 |
| *Escherichia coli* ATTC 8739 | 7 | 7 | 7 | 7 | 8 | 7 | 8 | 10 | NT | 0 |
| *Bacillus megaterium* DSM 32 | 7 | 0 | 7 | 7 | 0 | 0 | 0 | 20 | NT | 0 |
| *Pseudomonas aeroginosa* ATTC 9027 | 7 | 0 | 7 | 7 | 7 | 7 | 0 | 10 | NT | 0 |
| *Klebsiella pneumoniae* ATCC 13883 | 7 | 7 | 7 | 7 | 8 | 7 | 0 | 15 | NT | 0 |
| *Enterococcus faecalis* ATCC 15753 | 7 | 8 | 0 | 0 | 0 | 0 | 0 | 16 | NT | 0 |
| *Salmonella typhimurium* ATCC 13311 | 0 | 7 | 7 | 7 | 0 | 0 | 0 | 13 | NT | 0 |
| *Serratia* spp. (isolated in KSU) | 0 | 7 | 9 | 7 | 7 | 0 | 8 | 18 | NT | 0 |
| *Candida albicans* ATTC 10194 | 7 | 7 | 0 | 7 | 0 | 7 | 8 | NT | 18 | 0 |

**a** = Ethyl acetate; **b** = Methanol; **c** = Acetone; **d** = Hexane; **e** = Diethyl ether; **f** = Chloroform; **g** = Ethanol; **Con**. = Control, **Amp** = Ampicillin 10 mcg, **Nst** = Nystatin 30 mcg, Standards = Standard antibiotics, NT = Not tested.

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(*Received*: 31 December 2007; *Accepted*: 26 August 2008) AJC-6790