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In vitro* Antibacterial Activity of *Nigella sativa* Against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Bacillus cereus

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Abstract: Antimicrobial activity of *Nigella sativa* against *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 19615), *Escherichia coli* (ATCC 25922) and *Bacillus cereus* (ATCC 11778) were investigated. The seed extracts were prepared by the methanolic wetting extraction method. Filter paper discs impregnated with varying concentrations of *N. sativa* extract were tested by the disk diffusion method. The inhibition zones of the Mueller Hinton agar in different extract concentration showed that at 25 mg/20 μ L, 50 mg/20 μ L and 100 mg/20 μ L, the inhibition zones increased accordingly in *S. aureus* and *P. aeruginosa*. However, *N. sativa* was found to be inactive against ESBL producers (*E. coli*, *K. pneumoniae* and *B. cereus*).

Key words: Methanolic extracts, ESBL producers, gram-positive bacteria, gram-negative bacteria, alternative antimicrobial agents

INTRODUCTION

Nigella sativa (*N. sativa*) is an annual flowering plant, native to southwest Asia. The *N. sativa* seed is known as kalonji (Hindi), habbah Albarakah (Arabic) or siyah daneh (Persian). In English it is called fennel flower or sometimes just referred to as Nigella or black seed. The seed extracts and essential oil has a broad pharmacological effects such as anti-diabetic, spasmolytic and bronchodilator, antioxidant, hepatoprotective and also showed both *in vitro* and *in vivo* antimicrobial effects (http://en.wikipedia.org/wiki/Nigella_sativa).

The crude extracts of *N. sativa* were reported to have a promising effect on multi-drug resistant *S. aureus* (Dadgar *et al.*, 2006), *P. aeruginosa* and *Candida albicans* (Mashhadian and Rakhshandeh, 2005), *Shigella* spp., *Vibrio cholerae* and *E. coli* (Ferdous *et al.*, 1992).

Production of β lactamases is the most common mechanism of resistance among the Gram-negative bacteria (Philippon *et al.*, 1989). The vast majority of strains expressing these enzymes belong to the family Enterobacteriaceae like *K. pneumoniae* and some *E. coli* strains. β -lactam antibiotics are the most common treatment for bacterial infections. Extended-Spectrum β Lactamases (ESBL) have become a widespread serious problem and several aspects of them are worrying. The potential use of alternative antibiotics in drug-resistant bacteria from various plant extracts have been studied by

many researches. To document the antibacterial effects of *N. sativa*, both gram positive and gram negative bacteria were tested.

MATERIALS AND METHODS

***Nigella sativa* seeds:** The seeds were purchased from a local herbal shop in Kuala Lumpur, Malaysia.

Extraction method: A modification of reflux extraction and wetting procedure by Mashhadian and Rakhshandeh (2005) was used. Six hundred gram of *N. sativa* seeds in 1500 mL of methanol (HmbG Chemicals, Germany) were incubated for one week at 25°C with at least 5 times vibration per day. The extracts were filtered using Whatman filter paper and evaporated using rotary distillation apparatus. The extracts were further dried in a 50°C oven for 24 h and finally kept at 4°C until further testing.

Concentrations of extracts: Three different concentrations, 100, 50 and 25 mg mL⁻¹ in 10% dimethyl sulfoxide (DMSO) were prepared using vortex mixture. Whatman AA paper discs were injected with 20 μ L of different *N. sativa* extracts using a micropipette and were dried in the Gelman biosafety cabinet for 30 min. Negative control disc were prepared using a 10% DMSO. Commercial antibiotic discs were included as controls.

Bacterial strains: The bacterial strains used were *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 19615) and *Bacillus cereus*, purchased from Culti-Loops, Oxoid and kept at the Microbiology Laboratory, Universiti Teknologi Mara (UiTM), Malaysia. All the bacteria were cultured on Nutrient Agar (NA) (Merck). Inoculum were prepared in 5 mL Mueller Hinton broth (BD) with 3 to 5 colonies of each bacterial strains. The inoculum were incubated at 35°C for 2-3 h to get an approximately close to 0.5 McFarland standard for susceptibility testing (National Committee for Clinical Laboratory Standards, 1993).

Antimicrobial Susceptibility Testing (AST) by NCCLS method: Mueller Hinton plates were cultured with a standardized inoculum of each bacterial strain using sterile cotton swabs dipped into the adjusted suspension. Whatman AA paper discs impregnated with different *N. sativa* extracts were carefully placed on the seeded plate. The plates were incubated aerobically at 37°C and examined for zones of inhibition after 24 h.

RESULTS AND DISCUSSION

Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth *in vitro*. This ability may be estimated by the diffusion method.

In this study, we investigated the antibacterial effects of aqueous, methanolic seed extracts on standard gram positive and gram negative bacterial strains (Table 1). The best inhibition was seen at 100 mg mL⁻¹ *N. sativa* concentration in *S. aureus*. Similar inhibitory effects on *B. cereus* was observed at much lower concentration (50 mg mL⁻¹). When β lactamase producing *K. pneumoniae* and *B. cereus* were tested, large zones of inhibition are produced with a heaped-up, clearly defined edge; these are readily recognizable when compared with the sensitive control, however, regardless of the size of the zone of inhibition, they should be reported as resistant (Fig. 1). The end-point of inhibition is judged by the naked eye at

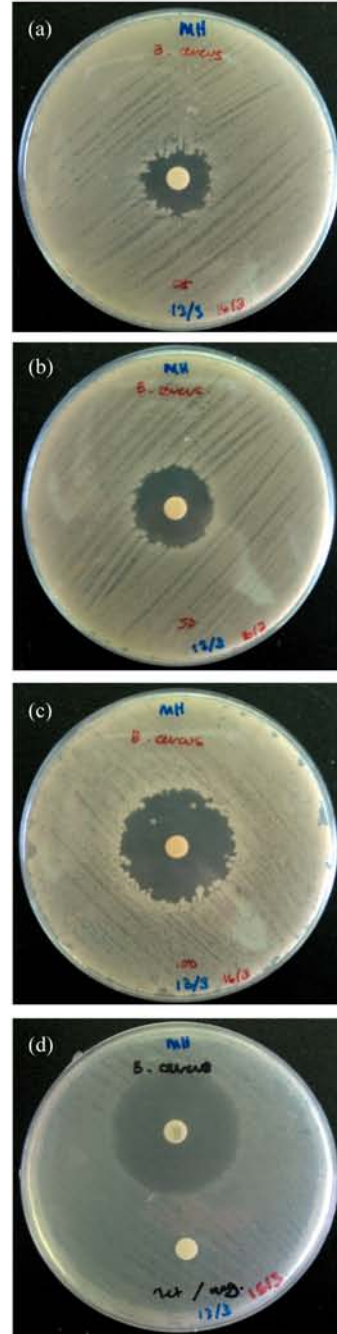


Fig. 1: Picture of zone inhibitory of *N. sativa* extract against *Bacillus cereus* (ATCC 11778). (a) Plate with 25 mg/20 µL of *N. sativa* extract. (b) Plate with 50 mg/20 µL of *N. sativa* extract. (c) Plate with 100 mg/20 µL of *N. sativa* extract. (d) Plate with tetracycline 30 µg disk as positive control (+ve) and 10% DMSO as negative control (-ve)

Table 1: Inhibition zone (mm) of methanol extract of *N. sativa* at various concentrations on selected ATCC bacterial strains

Bacteria tested	Relationship between extract concentration and diameter (mm)		
	25	50	100
	----- (mg mL ⁻¹) -----		
<i>S. aureus</i> (ATCC 25923)	14	17	25
<i>E. coli</i> (ATCC 25922)	-	8	9
<i>K. pneumoniae</i> * (ATCC 19615)	8	10	13
<i>P. aeruginosa</i> (ATCC 27853)	8	9	10
<i>Bacillus cereus</i> * (ATCC 11778)	10	18	21

*: β lactamase producer

the edge where the growth starts, but there are exceptions like these heaped-up growth characteristics. Tetracycline and 10% DMSO was used for quality check and negative control, respectively, on the MHA.

N. sativa has weak antibacterial activity on *E. coli* (9 mm) and *P. aeruginosa* (10 mm) at 100 mg mL⁻¹.

In this preliminary study, Minimum Inhibitory Concentration (MIC) of the extracts was not carried out, however the zone diameters of five bacteria were reported here. This study showed that the methanol extract of *N. sativa* seeds had the best antimicrobial activity to *S. aureus* compared to its activity on the gram-negatives. *K. pneumoniae* and *B. cereus* were resistant but *E. coli* and *P. aeruginosa* were weakly sensitive to this seed extract.

P. aeruginosa is an ESBL producer. ESBLs have become a widespread serious problem and several aspects of them are worrying. These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. They compromise the activity of wide-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients. The continued emergence of ESBLs presents diagnostic challenges to the clinical microbiology laboratories. In Malaysia, 19% of *E. coli* and 27% of *K. pneumoniae* are ESBL producers (Rahizan *et al.*, 1998). A detailed study on the possibility of extending the usefulness of *N. sativa* on the non-ESBL *K. pneumoniae* should be further investigated to provide more information including MIC values.

The finding of multi-resistance in community *S. aureus* in Malaysia is a concern. There is a particularly high rate of resistance to fucidic acid (11.8%) which is an antibiotic that is used to treat MRSA in the Malaysian hospitals (Lim, 2003). The broad spectrum of *N. sativa* activities against MRSA, anti-cestodes, anti-leishmania and antiviral therefore warrants further investigation.

N. sativa has other potentials. For example in Malaysia, other studies are being conducted to study the effect of the mixture of *N. sativa* and honey on the immune system and overall nutritional status (unpublished data) and an *in vivo* study effect of *N. sativa* on neurotransmitter activities in induced rats (unpublished data).

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