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### Effect of metal ions and drugs on antibacterial activities of *Nigella sativa* (L.) seeds

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#### ABSTRACT

The present study was conducted to find out the antibacterial activity of aqueous and organic extracts of *Nigella sativa* (L.) seeds against various bacterial pathogens, namely; *S. aureus*, *P. aeruginosa* and *E. coli*. Plant extract along with different elicitors such as metal ions and commercial drugs was also screened to see the change in efficacy and potency of the compounds. Among different extracts ethyl acetate extract proved maximum activity towards *Staphylococcus aureus* and *Pseudomonas aeruginosa* while minimum was observed against *Escherichia coli*. Ethanolic and methanolic extracts exhibited dose-dependent activity. Ciprofloxacin exhibited a synergistic effect with ethyl acetate extract giving zone of inhibition of 18.0mm against *S. aureus*. When 0.1% Cu was mixed with Ethyl acetate extract it proved 72.0% increase in the activity as it decreased the Minimum inhibitory concentration up to 1.25mg/ml from 4.5mg/ml. No growth was found in the Nutrient agar plate which was mixed with crude oil of *Nigella sativa* (L.) seeds.

**Keywords:** Antibacterial, *Nigella sativa*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, Antibiotics, Elicitors, Ethyl acetate extract, Thymoquinone.

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#### INTRODUCTION

*Nigella sativa* (L.) is a vegetal species of Ranunculaceae family, known as “black cumin seed”, “neguilla” or “ajemuz” that is widely cultivated in the Mediterranean region. Its seed have played an important role over the years in ancient Islamic system of herbal medicine and in Spain, where they have been traditionally used as folk medicine [1]. *Nigella sativa* seeds have shown several therapeutic effects such as prevention of cancer, antihypertensive effect, anti-

inflammatory, analgesic and antihistaminic actions [2-6]. *Nigella sativa* seed composition includes nutritional components such as carbohydrates (glucose, xylose, rhamnose, and arabinose), vitamins as thiamine, riboflavin, pyridoxine, niacin and folic acid, mineral elements (calcium, iron and potassium), proteins, alkaloids (nigellidine, nigellimine and nigellicine), 36%-38% fixed oil and 0.4%-2.5% essential oil [7].

## MATERIALS AND METHODS

### Preparation of plant extracts:

Air dried NS seeds were pulverized using a milling machine and extracted with Distilled water, Methanol, Ethanol and Ethyl acetate using suspension extraction and column extraction. For extraction through suspension method, equal amount of grinded powder of NS seeds were mixed with ten volumes of solvents (namely; water, methanol, ethanol and ethyl acetate) and were kept in dark at room temperature for 10 days, followed by filtration with Whatman filter paper No. 1, the filtrate was concentrated by evaporating the solvent and dried up to dried powder. For extraction through column method, plant material was mixed with equal amount of silica gel and filled in clean glass column, fitted with cotton at the bottom. Column was fixed vertically at column stand, wrapped with aluminum foil and filled at the top with ten volumes of the solvent. Knob was opened slowly and the extract was collected in a clean beaker kept at bottom. Extract was concentrated and dried up to dry powder and stored. Plant extract was used by dissolving in DMSO at concentration of 100mg/ml.

### Test Organisms:

Clinical isolates of pathogenic bacteria were used for bioassay studies. The test organism includes *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The isolates were maintained on freshly prepared nutrient agar plates and slants and keep in a refrigerator at 4<sup>o</sup> C until required for use. Single colony was transferred in sterile 50 ml of nutrient broth and incubated at 37 °C in shaker incubator at 140 rpm for 14 hrs. Bacterial cells were recovered by centrifugation and were suspended in sterile distilled water; concentration of pathogens was optimized by maintaining OD to 0.1 at 600 nm before use.

### Screening of bioactive compounds against various pathogens:

10.0 ml nutrient agar media was poured in a sterile Petri dish, 100 µl of test organisms were spread on the surface of media, wells were prepared with help of sterile borer and wells were aseptically filled by 30µl plant extracts along with positive (antibacterial compounds, namely; tetracycline at 50µg/ml) and negative control (autoclaved distilled water). Plates were incubated aerobically at 37 °C for 14 hrs. The diameters of zones of inhibition were measured.

### Determination of Minimum Inhibitory Concentration (MIC) of extracts:

This is carried out by double agar gradient plate method. Nutrient agar (5.0 ml) was poured into sterilized Petri dishes, leaving the plate in slanted position. After setting the media, another 5.0 ml of nutrient agar (along with plant extract; 5.0 mg/ml) was added to the plates to make the level unity; thus the plate contained an increasing concentration of plant extract along the diameter of the plate. Now the 70µl of prepared inoculums of cultures were spread. Plates were incubated in upright position at 37 °C for 14 hrs. Concentration gradient along with the diameter

was calculated for each mm. visible colonies were observed, distance was measured from top end and concentration of the compound was calculated as MIC.

### Effect of different elicitors on antibacterial properties of the compounds:

To see the effect of elicitors on the antibacterial activities of the plant extracts against various bacterial pathogens, 0.1% of different metal ions, namely; Magnesium, Iron, Copper, Lead and Zinc were added to the plant extract. Also, different antibiotics were added to the plant extracts to see the effect of plant extract in conjugation with antibiotics. Zone of inhibition was recorded for each variable to compare the effects. To see the effect of different commercial drugs on the activity of plant extracts against pathogens, several drugs (Ciprofloxacin, Tetracycline, Ampicillin, Metronidazole, Ofloxacin, Amoxicillin, Odoxil and Sparfloxacin; 50 $\mu$ g/ml each) were added with 60:40 ratio (30 $\mu$ l plant extract and 20 $\mu$ l drug per wells) and loaded into wells.

## RESULTS

Out of five different extracts of NS seeds, two extracts proved antibacterial activity against two strains and only one against all the three pathogens. Figure 1 shows a comparative analysis of the seed extracts against three bacterial pathogens.

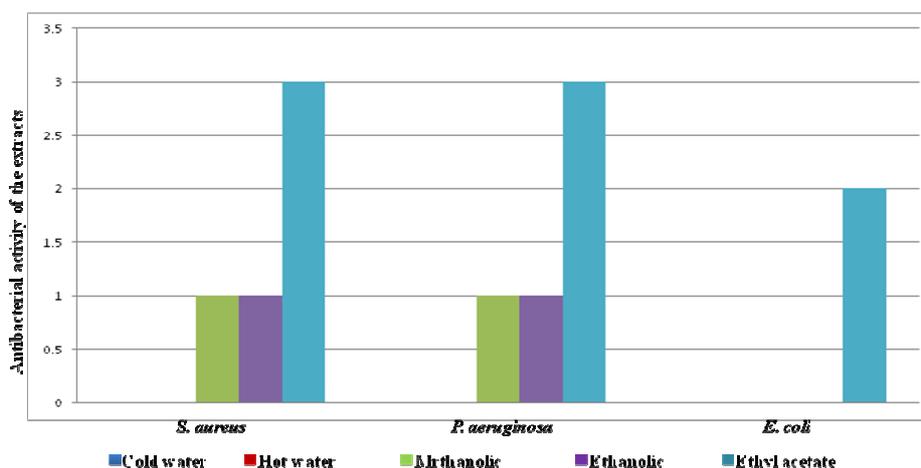
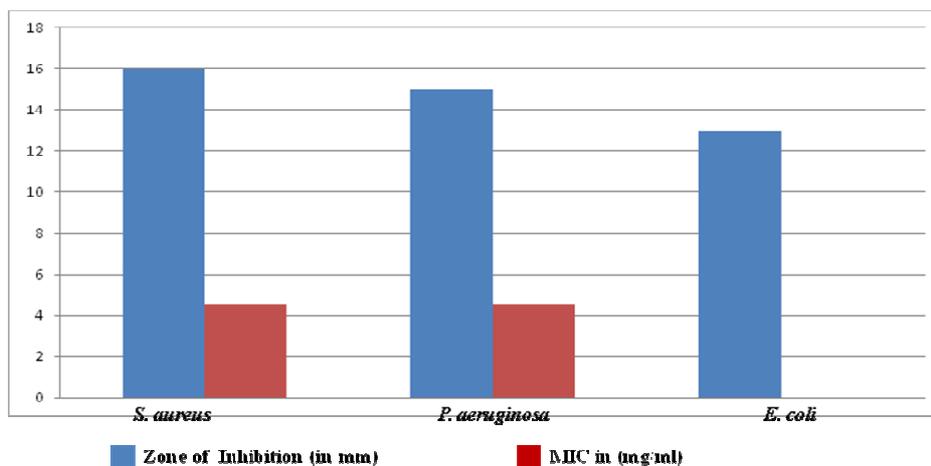


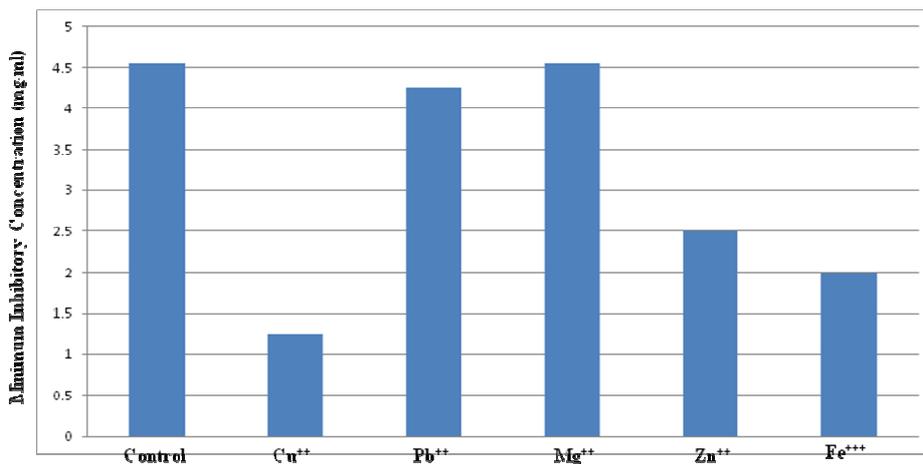
Fig. 1: Comparative analysis of different extracts of NS seeds against Bacterial pathogens.

Ethyl acetate was selected for extraction and determination of MIC against bacterial pathogens as out of five extraction procedure selected in the study, ethyl acetate proved best in terms of activity against all pathogens and higher zone of inhibition was recorded. Bioactive plant metabolites extracted with help of organic solvents, namely; methanol, ethanol and ethyl acetate proved better extraction solvent in comparison to aqueous extracts. Figure 2 shows comparative analysis of activity of the extracts (in terms of zone of inhibition; in mm) and potency of the compounds (in terms of minimum inhibitory concentration; mg/ml) against three bacterial pathogens, namely; *S. aureus*, *P. aeruginosa* and *E. coli*.



**Fig. 2: Comparative analysis of zone of inhibition and MIC of ethyl acetate extract of NS seeds against Bacterial pathogens.**

Ethyl acetate extract was supplemented with different elicitors (metal ions) to see the effect on potency of the compounds. Figure 3 shows a comparative analysis of different metal ions on MIC of ethyl extract of NS seeds against all the bacterial pathogens.



**Fig. 3: Comparative analysis of different metal ions on the activity of ethyl acetate extracts of NS seeds.**

Ethyl acetate extract of NS seeds was supplemented with different drugs to see the effect of plant metabolites in conjugation with drugs. Data shown below in Figure 4 suggests an increased activity of the compounds along with drugs.

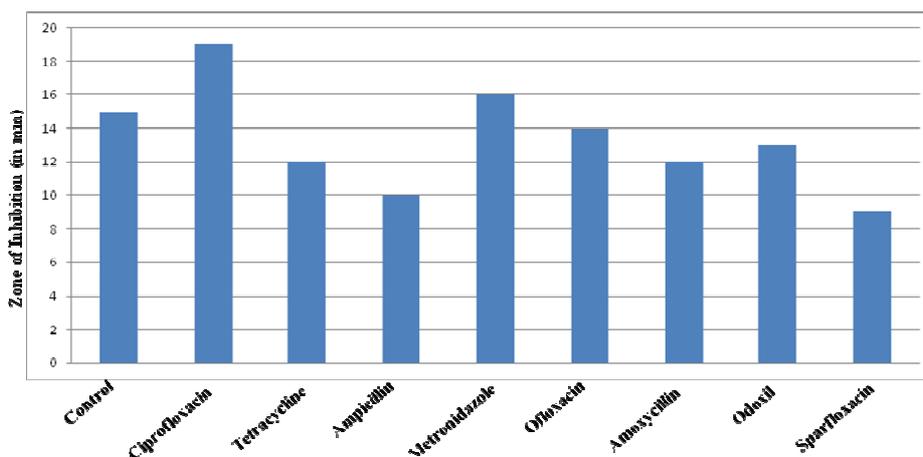


Fig. 4: A comparative analysis of different drugs along with ethyl acetate extract against pathogens.

## DISCUSSION

Herbal medicines are a valuable and readily available resource for primary health care and complementary health care systems. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have to be discovered, though large numbers of plants are constantly being screened for their antimicrobial effects. These plants may prove to be a rich source of compounds with possible antimicrobial activities but more pharmacological investigations are necessary.

*Nigella Sativa* seed ethyl acetate extract gave zone of 16.0mm, 15.0mm and 13.0mm against *S. aureus*, *P. aeruginosa* and *E. coli* respectively, while methanolic and ethanolic extracts gave positive results against *S. aureus* and *P. aeruginosa*, only with less zone of inhibition. Aqueous extracts of NS seeds failed to show positive result against any of the pathogen selected in the study (Fig. 1). Out of the three pathogens selected in the study, *E. coli* was most resistant in comparison to *S. aureus* and *P. aeruginosa*, as it showed less zone of inhibition and maximum MIC value for ethyl acetate extracts; while limited or no activity for methanolic and ethanolic extracts (Fig. 2). Copper ion was found best elicitor among all the metal ions selected in the study to enhance the activity of the plant extract against all the bacterial pathogens, followed by Iron and Zinc (Fig. 3). The effect of various elicitors on the antibacterial activity of various extracts was screened in the present research work as has been reported by [8]. Ethyl acetate extract of NS seeds proved enhanced activity in conjugation with Ciprofloxacin and Metronidazole, while other drugs showed less elicitive response when added to the extract (Fig. 4).

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