Effect of Metal Ions and Drugs on Antibacterial Activities of Nigella Sativa (L.) Seeds.

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Article ID: WMC002074  
Article Type: Original Articles  
Submitted on: 10-Aug-2011, 06:44:17 AM GMT    Published on: 10-Aug-2011, 08:38:12 PM GMT  
Article URL: http://www.webmedcentral.com/article_view/2074  
Subject Categories: AYURVEDIC MEDICINE  
Keywords: Antibacterial, Nigella Sativa, Staphylococcus Aureus, Escherichia Coli, Pseudomonas Aeruginosa, Antibiotics, Elicitors, Ethyl Acetate Extract, Thymoquinone.  
How to cite the article: Mishra R P. Effect of Metal Ions and Drugs on Antibacterial Activities of Nigella Sativa (L.) Seeds. . WebmedCentral AYURVEDIC MEDICINE 2011;2(8):WMC002074

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Author(s): Mishra R P

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.

Methods and Materials

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 power 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 40 C until required for use. Single colony was transferred in sterile 50 ml of nutrient broth and incubated at 37 oC 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, 70 % of test organisms were spread on the surface of media, wells were prepared with help of sterile borer and wells were aseptically filled by 30 % plant extracts along with positive (antibacterial

Introduction

Nigella sativa (L.) is a vegetal species of Ranunculaceae family, known as ?black cumin seed?, ?neguila? 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 (El-Naggar et al., 2010). Nigella sativa seeds have shown several therapeutic effects such as prevention of cancer, antihypertensive effect, anti-inflammatory, analgesic and antihistaminic actions (Swamy and Tan, 2000; Tahir et al., 1993; Abdel-Fattah, 2000; Chakravarty, 1993 & Khan, 1999). 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 (Hosseinzadeh and Parvardeh, 2004).
acetate proved better extraction solvent in comparison to organic solvents, namely; methanol, ethanol and ethyl acetate. Bioactive plant metabolites extracted with help of these solvents were screened in the present research work as has been reported by Auda et al., (2009). Ethyl acetate proved best in terms of activity against all the pathogens and higher zone of inhibition was recorded. Out of five extraction procedure selected in the study, ethyl acetate proved best in terms of activity against all the bacterial pathogens, namely; S. aureus, P. aeruginosa and E. coli.

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. 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.

**Discussions and Conclusion**

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 Auda et al., (2009). 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).
References

Illustrations

Illustration 1

Fig. 1: Comparative analysis of different extracts of NS seeds against Bacterial pathogens.
Illustration 2

Fig. 2: Comparative analysis of zone of inhibition and MIC of ethyl acetate extract of NS seeds against Bacterial pathogens.
Illustration 3

Fig. 3: Comparative analysis of different metal ions on the activity of ethyl acetate extracts of NS seeds.
Illustration 4

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

Review 1

Review Title: Review for the article entitled

Posted by Dr. Muniappan Ayyanar on 12 Aug 2011 11:46:04 AM GMT

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Comment:
6. The authors can provide some information regarding the role of metal ions in the antibacterial activity of medicinal plants with previously published literature.

9. There is no tables and figures in the manuscript, but in the text the authors provided legend for four figures.

Competing interests: No

Invited by the author to make a review on this article? : No

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How to cite: Ayyanar M.Review for the article entitled [Review of the article 'Effect of Metal Ions and Drugs on Antibacterial Activities of Nigella Sativa (L.) Seeds. ’ by J].WebmedCentral 1970;2(8):REVIEW_REF_NUM888
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