Antibacterial Activity of Extract of Seeds of *Nigella Sativa* Linn

Chetana S.H[1], Sangeeta Sajjan[1], Padmaa. M. Paarakh[2,*] and Vedamurthy A.B[1]

[1]Department of Biotechnology, The Oxford College of Science, J.P.Nagar, I.Phase, Bangalore 560 078, Karnataka, India.
[2]Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore 560 078, Karnataka, India.

Summary

Extracts of seeds of *Nigella sativa* Linn (Ranunculaceae) were screened for their *in vitro* antibacterial activity by agar diffusion method in comparison with standard antibiotic ampicillin, tetracycline, streptomycin, gentamycin and levofloxacin. The antibacterial activity of hexane, chloroform, methanol and aqueous extract of seeds of the plant were studied using *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* (Clinical isolate, Bacteria) as test organism. All the extracts were effective against all the four microorganisms. Only levofloxacin showed a higher zone of inhibition while all other standard antibiotics had a zone of inhibition less than the extracts of *Nigella sativa* indicating that the plant can fight these organisms effectively and it could be better alternative to the modern medicine.

Key words: *Nigella sativa* Linn, *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, in vitro* antibacterial activity, clinical isolate.

*** Correspondence Author:**
Dr. Padmaa M Paarakh
Department of Pharmacognosy
The Oxford College of Pharmacy
J.P.Nagar, I. Phase
Bangalore 560078
padmaparas@hotmail.com
Mobile: 00 91 9880681532

Introduction

Herbal medicines have been used for the cure of various diseases/physiological conditions. These natural products are also used for elimination of human ailments. Plants have contributed more than 7000 compounds being used today to treat diseases like cancer, hormonal imbalances, contraceptive, antibiotics, diuretics, heart diseases etc. There is a tremendous increase in search of antimicrobial plant extracts due to the fact that the resistance offered against antibiotics by the microorganism, in short the effective life span of any antibiotic is limited[1]. One such plant which has number of traditional uses is *Nigella sativa* Linn.

Nigella sativa Linn (Ranunculaceae), which is well known as black cumin, is a small elegant annual herb cultivated in Punjab, Himachal Pradesh, Gangetic plains, Bihar, Bengal, Assam, Maharashtra, Syria, Lebanon, Israel and Southern Europe[2, 3]. In the traditional system of medicine, the seeds are used as bitter, pungent, aromatic, appetizer, stimulant, diuretic, emmenagogue, galactogogue, anthelmintic, acrid, thermogenic, carminative, anodyne, deodorant, digestive, constipating, febrifuge, expectorant, purgative, abortifacient, in cough, jaundice, paralysis, abdominal disorders, diarrhea, dysentery, intrinsic hemorrhage, amenorrhea and dysmenorrheal[4]. The plant is reported to possess antitumor activity, immunomodulatory, antiulcer, antioxidant, galactogoue, anti-inflammatory, antiimplantation, anthelmintic, hepatoprotective, cardiovascular, CNS depressant and antineoplastic activities[5, 6]. There is report on the antimicrobial activity of seed oil[7, 8, 9]. Since there is no report on the antimicrobial activity of extract of the seed of *Nigella sativa*, an attempt was made to evaluate the antibacterial activity of hexane, chloroform, methanol and aqueous extracts of the plant by agar diffusion method using *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* (Clinical isolate, Bacteria) as test organism.

Materials and methods

Plant material:
Nigella sativa Linn seeds were collected from Gulbarga, Karnataka and authenticated by Central Council for Research in Ayurveda and Siddha, Bangalore. A voucher specimen has been preserved in our Department.

Extraction procedure

Shade dried seeds (470 g) were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (soxhlet). The extraction was done with different solvents in their increasing order of polarity such as hexane, chloroform, methanol and water. Each time the marc was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The yield was found to be 2.36, 1.26, 4.67 and 9.26% w/w with reference to the air dried plant. The dried extracts were dissolved in dimethyl formamide at a concentration of 5 mg/ml

Microorganisms and media
Gram positive bacteria: *Staphylococcus aureus*
Gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, Klebisella pneumoniae*
Bacterial isolates are the clinical isolate obtained from samples collected from the patients under treatment at The Bangalore Institute of Oncology, Bangalore. The bacterial stock cultures were maintained on Muller Hinton agar and stored at 4^0C.

Antibacterial activity:

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotics viz., ampicillin, tetracycline, streptomycin, gentamycin and levofloxacin *in vitro* by disc diffusion method[10] using *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* (Clinical isolate, Bacteria) as test organism. Muller Hinton agar was prepared, sterilized and

poured into petriplates up to a depth of 3 mm. The organisms were suspended in saline and 500 µl of organisms were spread on these plates on which wells were made using an 8 mm cork borer. To each well, 100 µl of each extracts were added and plates were incubated at 37^0C for 24 hrs for bacteria. After incubating for 24 hrs, the results were recorded by measuring the zone of inhibition surrounding the well. The experiments were done in triplicate.

Result and Discussion

The results of antibacterial activities are given in the Table 1 and 2. From the Table 1 and Fig 1, it is very clear that all the extracts have shown antibacterial activity against all the tested organisms. Hexane and methanol extracts were very effective followed by aqueous extract. While chloroform extract showed least activity against the entire tested organism. Susceptibility of these test organisms to traditional antibiotics was done using standard antibiotics such as ampicillin, tetracycline, streptomycin, gentamycin and levofloxacin. The zone of inhibition of the standard antibiotics against the test organism was measured and the results are given in Table 2 and Fig 2.

Table 1: Antibacterial activity of different extracts of seeds of *Nigella sativa* against microorganisms

Extracts	**Gram Negative Bacteria**			**Gram Positive Bacteria**
	E.coli	***P.aeruginosa***	***K.pneumoniae***	***S.aureus***
Hexane extract	20	19	17.5	19
Chloroform extract	13.5	11.5	11.5	12
Methanol extract	20.5	20	19	16
Aqueous extract	18	19.5	18	19

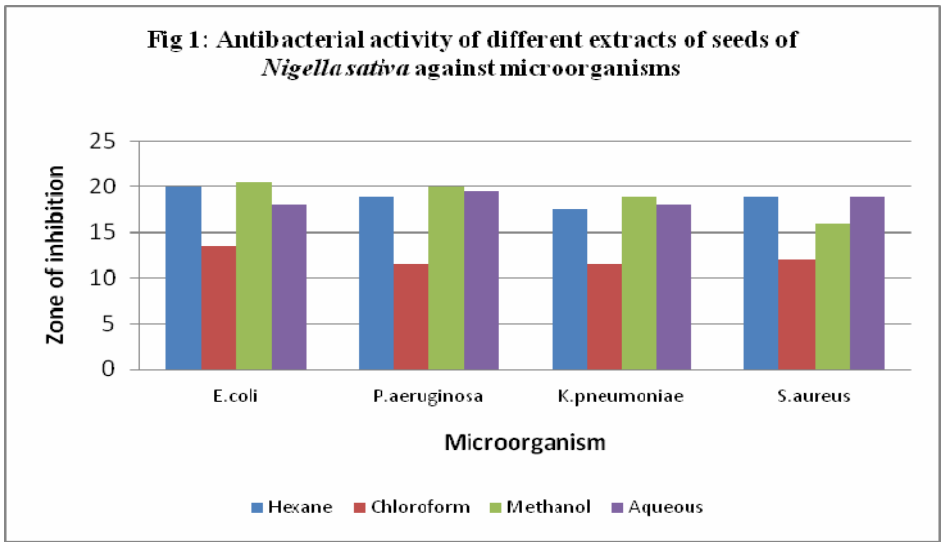
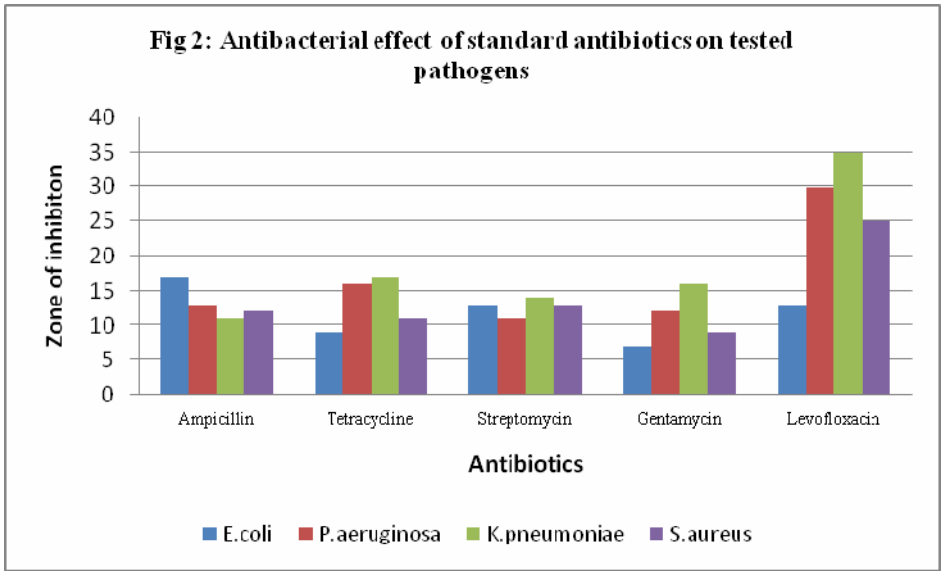


Table 2: Antibacterial effect of standard antibiotics on tested pathogens

Antibiotics	Gram Negative Bacteria			Gram Positive Bacteria
	E.coli	***P.aeruginosa***	***K.pneumoniae***	***S.aureus***
Ampicillin	17	13	11	12
Tetracycline	09	16	17	11
Streptomycin	13	11	14	13
Gentamycin	07	12	16	09
Levofloxacin	13	30	35	25



E.coli: Standard antibiotic ampicillin showed maximum zone of inhibition followed by streptomycin and levofloxacin. While tetracycline and gentamycin showed less zone of inhibition.

P.aeruginosa: Standard antibiotic levofloxacin showed maximum zone of inhibition followed by tetracycline. While streptomycin, gentamycin and ampicillin showed less zone of inhibition.

K.pneumoniae: Standard antibiotic levofloxacin showed maximum zone of inhibition followed by tetracycline, gentamycin and streptomycin. While ampicillin showed less zone of inhibition.

S.aureus: Standard antibiotic levofloxacin showed maximum zone of inhibition followed by streptomycin, tetracycline and ampicillin. While gentamycin showed less zone of inhibition.

It can be concluded that the plant extract possess antibacterial activity against test organism used. Some of the extracts were more effective than traditional antibiotics to combat the pathogenic microorganisms studied. This possibly means that the compound responsible for the antibacterial activity is present in each extract at different

concentrations. The chance to find antibacterial activity was more apparent in methanol and hexane extracts than in chloroform and aqueous extracts. The extracts were found to be effective against Gram negative (*E.coli, K.pneumoniae, P. aeruginosa*) pathogens when compared to Gram positive (*S.aureus*) pathogen.
With references to the standard antibiotic tests, the seed extracts showed a higher zone of inhibition except for antibiotic levofloxacin.
Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antibacterial activity. This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat diseases.

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