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Effect of *Nigella sativa* seeds extracts on clinically important bacterial and fungal species

Abstract

Nigella sativa is a medicinal plant proven to have curing potential in many diseases. The present study was aimed to investigate the antibacterial and antifungal activities of *N. sativa* seeds extracts prepared in methanol, ethanol and water. The results of plant extracts were found to be promising as compared to culture sensitivity results of the antibiotics. Methanol extracts exhibited maximum activity against *Staphylococcus aureus* with 20 mm zone of inhibition. Ethanol extracts exhibited optimum activity against *Proteus vulgaricus* and *Pseudomonas aeroginosa* with 21mm zone of inhibition. Water extract showed highest activity against *Staphlococus aureus* (16mm zone of inhibition) and lowest activity against *P. vulgaricus* with 4 mm zone of inhibition. Against fungal isolates methanol extracts exhibited maximum activity against *Penicilium digitatum* (20mm ZI), whereas the ethanol extracts showed optimum activity against *P. digitatum*. Water extract showed highest activity against *Rhizopus stolonifer* with ZI recorded is 12mm and showed least activity against *Penicilium digitatum* with ZI observed is 6mm. It was concluded that *N. sativa* seeds have significant antibacterial activity against gallbladder and infantile septicemia pathogens.

Keywords: *nigella sativa*, gallbladder, infantile septicemia, antibacterial, antifungal activity

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Introduction

Salmonella typhi and *Salmonella paratyphi* A are the main causative agents of enteric fever.[1,2] The infection is common in those areas where unhygienic conditions prevailed. According to estimation round about 27 million people become victim of typhoid annually, of this 27 million 200000 people die.[3] More than 95% of patients can be recovering by providing appropriate therapy. But study suggests that 2-5% of typhoid patients got chronic gallbladder infection. Gallbladder infection may be due to bile or directly from blood. Salmonella specie is acquiring resistance to antibiotics such as ciprofloxacin, ampicillin, cotrimoxazole, chloramphenicol and ceftriaxone, which has made difficult the management and treatment of enteric fever.[1]

According to estimation, 4 million infants die every year around the world, out of which 1 million die because of infantile sepsis.[4,5] Major causative agents of the infantile sepsis are *Pseudomonas, E. coli, Proteus, Staphylococcus aureus* and *Klebsilla*, out of these *Pseudomonas* and *E. coli* have developed multi drug resistance against cefotaxime, ceftrioxone, ceftzidine, gentamycin, amoxicillin and ampicillin.[5] Mortality rate of neonatal septicemia is increasing due to emergence of antimicrobial drug resistance which is considered to be a challenge for researchers.[5,6]

Nigella sativa is a medicinal plant believed to have antitumor, antidiabetic, diuretic, gastroprotective, CNS depressant, antispasmolytic, anti-inflammatory, antioxidant, antimicrobial, anticonvulsant, antiurolithatic, antinociceptive, anxiolytic, hepatoprotective, nephroprotective, antihelminthic and immunomodulatory activitie.[7] *N. sativa* seeds extracts is used to treat coughs and remove renal stone and inhibit the growth of cancerous cells. It is used for the treatment of abdominal pain, polio and diarrhea. It also has anti-inflammatory and antioxidant activities.[8] The oil of *Nigella sativa* have antihelminthic, antinematodal, Antischistosomal, antimicrobial and antiviral activities.[9,10]

Materials and methods

Collection and processing of the seeds

The seeds of *N. Sativa* were purchased from the local herbal market in Khyber bazaar Peshawar. The collected seeds were identified from the department of Botany, University of Peshawar. The healthy seeds were washed three times with distilled water and dried at 40°C overnight. After drying, the seeds were grounded into powder by an electric grinder and preserved.[11]

Preparation of extracts

A 100g of powdered seeds material was dissolved in 1liter of methanol, ethanol and distilled water and kept for 24h at 25°C. After 24h, the material was filtered and the filtrate was placed on water bath at 60°C for the evaporation of extra solvents. The extracts obtained were refrigerated at 4°C for further studies.

Test bacterial and fungal cultures

Various bacterial species namely *Salmonella typhi*, *Salmonella paratyphi A*, *Escherichia coli, Staphylococcus aureus, Klebsilla pneumonia, Pseudomonas aeruginosa* and *Proteus vulgaricus, Staphlococcus epidermidis, Klebsiella oxytoca* and *Proteus mirabilis* were isolated from the infantile sepsis and gallbladder patients in LRH, Peshawar. Fungal species including *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus stolonifer, Penicillium digitatum* and *acremonium alternatum* were provided by the laboratories of LRH, Peshawar and Microbiology laboratory of Abasyn University Peshawar.

Bacterial strains and antibiotics susceptibility test

The effects of some commonly prescribed antibiotics were investigated against the test bacterial strains. For antibacterial assay, disc diffusion method was used on MHA. Standard antibiotics like

Amoxycillin (20µg), Gentamicin (10µg), Clavulanic acid (10µg), Amikacin (30µg), Ceftrixone (30µg), Ciprofloxacin (5µg) and Vancomycin (30µg) discs were investigated for their antibacterial activities against all the tested bacteria. The antibiotics discs were placed on the inoculated agar plates and the plates were incubated at 37°C for 24h followed by the measurement of inhibition zone.

Antibacterial activity

Agar well diffusion method was carried out to evaluate the antibacterial activity. The nutrient agar plates were prepared and test bacterial strains were inoculated on it. Then by means of 6mm borer wells were produced on agar plates. From each extract, 100µl was transferred through micropipette into the wells in the inoculated agar plates.[12] For better diffusion of the extracts before incubation; the plates were placed at 4°C for 3-4h.[11] The bacterial inoculated plates were incubated at 37°C for 24h. After the completion of incubation period, zone of inhibition (mm) was measured. Each concentration of the extract was assessed for antibacterial activity in triplicate and their mean zone of inhibition was taken.[13] A 50µl DMSO was used as negative control.

Antifungal activity

The potato dextrose agar (PDA) plates were prepared and the fungal strains like *A.* fumigatus, *A. niger, R. stolonifer, P. digitatum* and *A. alternatum* were spread on the PDA. A sterile 6mm diameter cork borer was used to made holes in the inoculated plates.[14] 50µl from each extract was applied through micropipette into the wells in the inoculated PDA plates.[12] For better absorption of the extracts before incubation the plates were placed at 4centigrade for 3-4hours.[11] The fungal inoculated plates were incubated at 25°C for 48h. After the completion of incubation period zone of inhibition was measured in mm by means of scale. Each concentration of the extracts was assessed for antifungal activity in triplicate and their mean zone of inhibition was taken.[13]

Statistical data analysis

Statistical analysis was performed using Microsoft Excel. All experiments were carried out in three different sets with each set in triplicates. The data are expressed as mean ± SEM (standard error of the mean).

Results

Culture sensitivity profile of bacterial and fungal species

In this study, different antibiotics were used to assess the susceptibility pattern of the bacterial strains (Table 1). Results showed that the Ciprofloxacin antibiotic exhibited the highest zone of inhibition (28mm) against *E. coli* (Infantile sepsis isolate), whereas a maximum zone of inhibition was recorded against *Salmonella typhi* (Gallbladder isolate) by Vancomycin. In case of fungal strains, *Acremonium alternatum* was found to be the most sensitive by Nystatin followed by *Rhizopus stolonifera* and *Penicilium digitatum.*

Table 1 Culture sensitivity results of bacterial and fungal species by different antibiotics

Microorganism	CN	CRO	CA	CIP	AMC	AK	VN
	Diameter of inhibition zone (mm)						
Infantile sepsis isolates (bacteria)							
Escherichia coli	12±0.22	20±0.18	9±0.07	28±0.24	5±0.02	30±0.29	32±0.36
Staphylococcus aureus	21±0.25	9±0.10	12±0.14	13±0.10	2±0.01	8±0.09	13±0.14
Staphylococcus epidermidis	19±0.34	6±0.02	10±0.13	5±0.04	3±0.02	7±0.06	11±0.08
Klebsiella pneumonia	11±0.12	10±0.07	8±0.17	8±0.07	4±0.05	12±0.12	14±0.15
Klebsiella oxytoca	9±0.09	10±0.11	7±0.05	8±0.12	6±0.08	4±0.03	16±0.24
Proteus vulgaricus	19±0.14	14±0.12	13±0.12	20±0.35	3±0.01	17±0.18	26±0.21
Proteus mirabilis	17±0.28	14±0.04	15±0.22	19±0.42	4±0.02	16±0.14	25±0.32
Pseudomonas aeroginosa	23±0.13	10±0.16	12±0.26	21±0.28	2±0.02	8±0.08	10±0.07
Gallbladder isolates (bacteria)							
Salmonella typhi	13±0.26	8±0.03	10±0.32	5±0.02	7±0.01	11±0.09	19±0.19
Salmonella paratyphi A	10±0.41	7±0.02	12±0.06	ND	9±0.12	8±0.10	17±0.51
Fungal strains	**FZ**	**KZ**	**NT**				
Aspergillus niger	2±0.22	13±0.27	16±0.11				
Aspergillus fumigatus	11±0.22	14±0.13	12±0.09				
Rhizopus stolonifer	3±0.22	17±0.25	18±0.18				
Penicilium digitatum	2±0.22	ND±0.12	17±0.15				
Acremonium alternatum	1±0.05	15±0.21	19±0.23				

CN, Gentamicin; CRO, Ceftriaxone; CA, Clavulanic acid; CIP, Ciprofloxacin; AMC, Amoxicillin; AK, Amikacin; VN, Vancomycin; FZ, Fluconazole; KZ, Ketoconazole; NT, Nystatin

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Antibacterial and antifungal activity of plant extracts

Antibacterial activity of the *N. sativa* seeds extracts was determined against the selected bacterial species and ZI were measured as shown in Table 2. The ZI recorded for *E. coli* was 19, 18 and 12 mm for ethanol, methanol and water extract, respectively, which showed that *E. coli* is sensitive to the seeds extracts. The ZI measured for *S. aureus* was 19, 20 and 16mm for ethanol, methanol and water extracts, respectively, evidencing the potency of the extracts against *S. aureus*. Contrary to *S. aureus*, *S. epidermidis* showed resistance towards ethanol extract and the ZI observed was 3, 11 and 15mm for ethanol, methanol and water extracts, respectively. *K. pneumonia* also showed resistance towards ethanol extract and *K. oxytoca* and was found sensitive towards ethanol (12mm) and water (14mm) but was found resistant towards methanol with no detected ZI. *P. vulgaricus* was sensitive towards ethanol and methanol extracts but resistant towards water extract. *P. mirabilis* and *P. aeuroginosa* were found sensitive towards all the extracts with different ZI. Similarly, the investigated plant extracts also exhibited considerable antimicrobial effects against all the tested fugal strains and therefore different ZI.

Table 2 Antimicrobial and antifungal potentiality of N. sativa seeds extracts against different strains

Microorganism	Ethanol	Methanol	Water	DMSO
	Diameter of inhibition zone (mm)			
Infantile sepsis isolates (bacteria)				
Escherichia coli	19±0.13	18±0.17	12±0.12	ND
Staphylococcus aureus	19±0.26	20±0.43	16±0.27	ND
Staphylococcus epidermidis	3±0.03	11±0.09	15±0.17	ND
Klebsiella pneumonia	2±0.01	16±0.21	14±0.14	ND
Klebsiella oxytoca	12±0.21	ND	14±0.22	ND
Proteus vulgaricus	21±0.43	18±0.26	4±0.33	ND
Proteus mirabilis	18±0.16	14±0.11	13±0.63	ND
Pseudomonas aeruginosa	21±0.18	19±0.23	11±0.15	ND
Gallbladder isolates (bacteria)				
Salmonella typhi	14±0.19	11±0.08	10±0.37	ND
Salmonella paratyphi A	13±0.09	12±0.16	9±0.07	ND
Fungal strains				
Aspergillus niger	15±0.23	14±0.073	9±0.01	ND
Aspergillus fumigatus	17±0.27	19±0.36	7±0.03	ND
Rhizopus stolonifera	16±0.11	15±0.05	12±0.01	ND
Penicilium digitatum	18±0.34	20±0.29	6±0.01	ND
Acremonium alternatum	13±0.22	17±0.13	11±0.06	ND

Discussion

Due to irrational use of antibiotics, prolonged hospitalization and improper curing and preventive procedures to control the pathogenic microorganisms, infectious agents are acquiring resistance to commercial antibiotics.[15,16] Moreover the antibiotics are not so much effective and if effective they are either unavailable or expensive and also have certain level of toxic effects on human body.[17,18] Therefore, researchers are trying to discover such compounds in plants which are effective, less expensive and have no or less toxicity. In order to overcome the resistance problem and discover drugs which are effective, less expensive, less toxic and easily available we focused on medicinal plants and designed the current study by using *N. sativa* seeds extracts against Enteric fever is caused by *S. typhi and S. paratyphi* A. In most of the cases, Salmonella infection can be treated by proper therapy but occasionally Salmonella enter into gall bladder and cause serious gallbladder complications which are difficult to cure. Death rate from infantile sepsis is increasing because of the resistance problem acquired by pathogens towards available drugs like cefotaxime, ceftrioxone, ceftzidine, gentamycin, amoxicillin and ampicillin which is challenging task for the researchers. Therefore, our study aimed to evaluate the antibacterial and antifungal activities of a medicinal plant known as *N. sativa* against gallbladder, infantile sepsis patient's isolates and some clinically important pathogenic fungi.

Eight bacterial species were isolated from infantile septicemia patients and two species were isolated from gall bladder patients and then culture sensitivity profile was determined for these isolated strains. *K. pnemonia and K. oxytoca* showed resistance towards 86% of the antibiotics used. Similarly *P. auroginosa* exhibited 71%, *S. epidermidis* 71%, *S. typhi* 28%, *S. paratyphi* 28%, *P. vulgaricus* 14%, *P. miribilis* 14%, *E. coli* 42% and *S. aureus* 57% resistance against antibiotics applied in the study. Our results for culture sensitivity tests showed little variations with research performed by[19] showing that *P. aeroginosa* and *S. aureus* were 50% resistant, *S. typhi* 71.4%, *k. pnemonia* 85.7% and *E. coli and P. vulgaricus* 78.6% resistant to various antibiotics.[20] Proved that *Klebsilla* has 100%, 76.3%, 68.4%

and 28.7% resistant against Amoxicillin, gentamicin, ciprofloxacin and ceftriaxone, respectively. The results also indicate that *E. coli* showed 96%, 83.4%, 76.4% and 32.5% against Amoxicillin, gentamicin, ciprofloxacin and ceftriaxone, respectively. Similarly, resistance exhibited by Proteus specie against antibiotics used were Amoxicillin (95%), gentamicin (77.2%), ciprofloxacin (65%) and ceftriaxone (23.4%).

Ethanolic, methanolic and water extracts of the seeds were evaluated for their antibacterial activity. All the extracts showed significant results against the tested strains. Ethanol extract was proven to have more effective as compared to methanol and water extracts. Ethanol extract exhibited maximum activity against *P. auroginosa* with 21mm ZI and minimum activity against *K. pnemonia* with 2mm ZI. It was concluded that aqueous extracts have less activity as compared to ethanol and methanol extracts and the reason might be that water can only extract polar compounds while ethanol and methanol are amphiphilic in nature and can extracts organic and inorganic compounds both. Similarly all the three extracts showed promising results against other tested isolates and their activity was impressing as compared to the activity of commercial antibiotics. Our study concluded that *N. sativa* seed extracts prepared in ethanol, methanol and distilled water has antimicrobial activity against both bacterial and fungal strains isolated from gallbladder and infantile sepsis patients.

Competing interests

The author declares that there is no conflict of interests.

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None.

Author's contributions

SIAS performed the experimentation; SBS wrote the first draft and helped in giving critical view of manuscript writing. LS and MB designed the study, wrote the manuscript and carried out the statistical analysis. MS participated in the collection of different plant parts and helped in experimentation. SBS also participated in taxonomist assessment for plant identification and in writing manuscript. All authors read and approved the final manuscript.

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