International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 4; July 2018; Page No. 49-53

Antibacterial activity of solvent extracts of *Nigella sativa* seeds against selected bacterial strains

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Abstract
In recent years lot of attention is being diverted towards plant extracts as antimicrobial agent. In this context present study is aimed to evaluate antibacterial properties and phytochemicals in *Nigella sativa* seeds. The antibacterial activity of solvent extracts of *Nigella sativa* seeds were tested against gram positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Salmonella typhimurium*. Design consists of three replicates, positive and negative controls. Agar well diffusion method was used for culturing. Zone of inhibition was measured in millimetres. Phytochemicals screening was carried out using standard protocols. According to the results, against the bacteria *S. typhimurium* and *S. aureus* maximum inhibitory zone was exhibited by chloroform and acetone extract respectively and various secondary metabolites were reported. The current study showed the potentiality of *N. sativa* seeds for controlling bacterial growth and emphasizes its development in the form of a natural antibacterial agent for the control of pathogenic microorganisms.

Keywords: *Nigella sativa*, Plant extracts, antibacterial activity, ampicillin, phytochemicals

1. Introduction
In recent years use of commercial antimicrobial drugs against pathogenic microorganisms has increased extensively. Effective antimicrobials have been developed over the past years. Several reports show the development of antibiotic resistance of human pathogens to available antibiotics [1]. Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance, search for natural products with potential antimicrobial activity has increased [2].
Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that most of the plants are medicinal and are active against drug resistant pathogens [3]. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Hence, researchers have revived their concentration on botanical extracts because of their safer nature and various biologically active compounds isolated from plants are being used in herbal medicines with acceptable therapeutic index [4].
Since antiquity many plant species are reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, tripenes etc., which should therefore be utilized to combat the disease-causing pathogens [5,6]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action of the human body [7]. The use of plant and its products has a long history of incorporation in traditional medicine.
Worldwide attention has been shifted towards finding new drugs. Natural products can provide unique components of various biological functions which are indispensable for novel drug discovery [8]. Many products of medicinal plants prove to be very useful in reducing the adverse effects of various chemo therapeutic agents as well as prolonging longevity and achieving positive health care system [9]. The research for new therapeutic treatments for various disease conditions is expanding. In many poor countries plants are considered as very promising source of new compounds for drug discovery and development [10].
Against the common antibiotics many human pathogens has developed drug resistance which has necessitated a search for new antimicrobial substances from sources including plants [11]. Customers are more concerned about the pathogenicity and mortality rate of the product they use. Therefore, with the advancement of the technology, scientists are challenged to come out with new ideas of alternative and novel drugs to overcome the usage of microbial resistant drugs.

2. Materials and methods
2.1 Collections of test materials
Seeds of *Nigella sativa* were collected from an Ayurveda store in Coimbatore.

2.2 Preparation of seed powder and extracts
Seeds of *N. sativa* were collected, and air dried under shade. Dried seeds were powdered using an electric pulveriser. Fine powder was obtained by sieving. The powder was subjected to extraction [12, 13]. Acetone extraction was followed by chloroform extraction and ethanol extraction so that the powders were subjected to extraction with solvents of increasing polarity. The seed extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40^0C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further use. Antibacterial activity of the seeds of *N. sativa* was investigated**.**

2.3 Test microorganism
The bacterial strains used were the clinical isolates obtained from an authenticated source. The bacterial strains used were *Staphylococcus aureus* and *Salmonella typhimurium*.

2.4 Antibacterial assay
The activity of various solvent extracts of seeds of *N. sativa* on selected bacterial strains was assayed by agar well diffusion method.

2.5 Agar-Well diffusion method
2.5.1 Media preparation and its sterilization
For agar well diffusion, method of Murray *et al.* [14] later modified by Olurinola [15] was used. Antibacterial susceptibility was tested on solid media in petriplates. For bacteria Nutrient agar was used for developing surface colony growth.

2.5.2 Nutrient agar
Nutrient agar medium was prepared and poured on to the petriplates and was left on sterile surface until the agar has solidified. The plates were swabbed (sterile cotton swabs) with 24 h old culture of bacterial strains. Wells were made in each of these plates using sterile cork borer. Stock solution of each solvent extract viz., Acetone, Chloroform and Ethanol was prepared at a concentration of 1 mg/ml. About 50µl of different solvent extracts of the seeds of *N. sativa* was added using sterile syringe into the wells and allowed to diffuse at room temperature for 2 h. Ampicillin was used as positive antibacterial control and negative control was also maintained. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The antibacterial activity was determined by measuring the diameter of zone of inhibition formed around well [16]. Triplicates were maintained, and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

2.6 Statistical analysis
The antimicrobial data was interpreted by calculating standard deviation and mean of three replicates.

2.7 Phytochemical screening
Preliminary phytochemical screening of selected seed extract was carried out using the standard protocols.

2.7.1. Test for Alkaloids
- **Mayer's test [17]:** 1 ml of extract was treated with a drop or two of Mayer's test reagent along the sides of test tube and observed for the formation of white or cream coloured precipitate.
- **Wagner's test [18]:** 1 ml of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish brown colour precipitate.
- **Hager's test [19]:** 1 ml of extract was treated with 1 or 2 ml of Hager's reagent and observed for the formation of prominent yellow precipitate.

2.7.2. Test for Tannins
Ferric chloride test [20]: 0.5 g extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green or blue-green precipitate.

2.7.3. Test for Phenols
- **Ferric chloride test [21]:** The extract (50 mg) was dissolved in 5 ml of distilled water and treated with few drops of 5% ferric chloride and observed for the formation of dark green colour
- **Lead acetate test [22, 23]:** The extract (50 mg) was dissolved in 5 ml of distilled water and 3 ml of 10% lead acetate solution was added and observed for the formation of bulky white precipitate.

2.7.4. Test for Flavonoids
- **NaOH test [20]:** 1 ml the extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.
- **Lead acetate test [22, 23]:** Fifty milligram of the extract was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow coloured precipitate.

2.7.5. Test for Sterols
Liebermann-Burchard test [24]: The extract (50 mg) was dissolved in 2 ml of acetic anhydride. To this one or two drop of Conc. H_2SO_4 was added along the side of the test tube and observed for any colour change.

2.7.6. Test for Terpenoids
Liebermann-Burchard test [25]: A little of extract (50 mg) was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of Conc. H_2SO_4. Change of colour from pink to violet indicates the presence of terpenoids.

2.7.7. Test for Saponins
Foam Test: The extract (50 mg) or dry powder was diluted with distilled water and made up to 20 ml. The solution is vigorously shaken for 15 minutes and observed for the formation of 2 cm layer thick foam.

2.7.8. Test for Anthraquinones
Borntrager's test [26]: Extract (0.2 g) to be tested was shaken with 10 ml of benzene and then filtered. Five ml of the 10% ammonia solution was added to the filtrate, shaken and observed for the appearance of a pink, red or violet colour.

2.7.9. Test for Proteins
- **Ninhydrin test [27]:** Three drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of extract and observed for the present of characteristic purple colour.
- **Biuret test [27]:** Two ml of extract was treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol was added followed by excess of potassium hydroxide pellets and observed for the formation of pink ethanolic layer.

2.7.10. Test for Quinones

- **H_2SO_4 test [23]:** To 1 ml of extract, 1 ml of Conc. H_2SO_4 was added and observed for the formation of red colour.
- **HCl test [28, 29]:** To 1 ml of the extract, 5 ml of HCl was added and observed for the presence of yellow colour precipitate.

3. Results

Plants have been a source of medicine from time immemorial. This is because it holds many antibacterial, antiseptic, antibiotic properties. The antibacterial activity of plant extracts is mainly due to the presence of chemical compounds obtained from the plants. They are named as phytochemicals. In the present study antibacterial activity of seed extract of *N. sativa* was determined against different bacterial strains viz., *S. aureus* and *S. typhymurium* and results are presented in Table 1. Their activity is recorded as inhibition zone diameter measured in millimeter (mm). Ampicillin is used as positive control and a negative control was also maintained.

Table 1: Antibacterial potential of *Nigella sativa* seeds

Test organisms	Acetone extract	Chloroform extract	Ethanol extract	Positive control	Negative control
Staphylococcus aureus	8.3 ± 1.53	5.7 ± 1.15	3.7 ± 1.15	3 ± 1	0
Salmonella typhimurium	5 ± 1.73	9 ± 2	4.7 ± 1.15	3.6 ± 0.57	0

Against the bacteria *S. typhimurium* a maximum inhibitory zone of 9 mm was exhibited by chloroform extract of *N. sativa* seeds. Followed by chloroform extract acetone extract provided a protection zone of 5 mm, thereby exhibiting moderate antibacterial activity against the test organism. The activity of chloroform extract was followed by ethanol extract of *N. sativa* indicating a zone of inhibition of 4.7 mm. Positive control showed the least inhibitory zone of 3.6 mm.

Among the three solvents acetone, chloroform and ethanol tested for its antibacterial activity, highest inhibitory zone of 8.3 mm was exhibited by acetone extract of *N. sativa* seeds against the test bacteria *S. aureus*. The activity of acetone was followed by chloroform which showed a zone of 5.7 mm. Minimum antibacterial efficacy was displayed by ethanol extract of *N. sativa* seeds recording a sensitivity zone of only 3.7 mm, which was comparatively equal to that of the standard antibiotic Ampicillin which showed an inhibitory zone of 3 mm.

Among the two organisms *S. typhymurium* has more resistant against seed extract of *N. sativa* than *S. aureus.* Chloroform extract of *N. sativa* seeds were more potent providing a sensitivity zone of 9 mm against *S. typhimurium* followed by acetone extract of *N. sativa* which showed an inhibitory zone of 5 mm against the same bacterium. Even the least active ethanol extract provided an inhibitory zone of 4.7 mm against *S. typhimurium* which was higher than that provided by the standard Ampicillin (3.6 mm).

Results of phytochemical screening are displayed in Table 2. *Nigella sativa* seed extract tested for its antibacterial activity against *S. aureus*, showed that acetone extract has high (8.3 mm) antibacterial potential and moderate antibacterial activity against *S. typhimurium* (5.0 mm). Therefore the extract was tested for its phytochemical constituents and it showed the presence of saponins, tannins, terpenoids, sterols and proteins.

Table 2: Phytochemicals present in the extracts of *Nigella sativa* seeds

Sl. No.	Constituents	*Nigella sativa* seed		
		Acetone extract	Chloroform extract	Ethanol extract
1	Alkaloids	-	-	+
2	Flavonoids	+	-	+
3	Sterols	+	+	+
4	Terpenoids	+	-	-
5	Anthroquinones	-	-	-
6	Phenols	+	+	+
7	Saponins	+	+	+
8	Tannins	+	+	+
9	Proteins	+	+	+
10	Quinones	+	+	+

'+' Detected, '-'Not Detected

Moderate antibacterial potential against the bacteria *S. aureus* and maximum activity against *S. thyphimurium* was exhibited by chloroform extract and its phytochemical analysis showed the presence of compounds such as flavonoids, saponins, tannins, terpenoids, phenol sterols, quinones and proteins. Minimum antibacterial activity against the bacteria *S. typhimurium and S. aureus* was exhibited by ethanol extract of *N. sativa* seeds and therefore studied for its phytochemicals and it showed the presence of phytoconstituents such as alkaloids, flavonoids, sterols, phenol, saponins, tannins, protein and quinones.

4. Discussion

An alarming increase in bacterial strains resistant to a number of antimicrobial agents demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Many plants have been investigated for antimicrobial activity and many plant extracts have inhibited growth of bacterial pathogens according to reports of Salman *et al.* [30]. In the present study among the two test organisms *S. typhimurium* which is a gram-negative strain showed more resistance against seed extracts of *N. sativa*, than the bacterium *S. aureus* which is a gram-positive strain. This may be because of the reason that Gram-positive bacterial strains are more susceptible to the extracts when compared to Gram negative bacteria.

In the present investigation it was noted that the zone of inhibition determined by agar well diffusion method varied with the different extracts, of the same seeds. It may be due to the solvent used for extraction, and the organism tested. The findings parallel to present study has been reported by Dahiya and Purkayastha [31] in which they have assessed an *in vitro* antibacterial activity of various solvents and water extracts of *Aloe vera*, neem, bryophyllum, lemongrass, tulsi, oregano, rosemary and thyme on 10 multi-drug resistant clinical isolates and found that results were solvent and organism dependent.

In the present study all the three extracts of *N. sativa* seeds has reported the presence of sterols, saponins and tannins in them. Callow [32] has reported that plant sterols are known to be

important for their cardiotonic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics. Saponins have the property of precipitating and coagulating red blood cells. Venkataswamy *et al.* [33] has reported that two groups of bacteria differ in their structure of cell wall. Ability of tannin to disintegrate bacterial colonies is hindered with bacterial cell wall. Medicinal plants which are rich in tannins are used to treat inflamed or ulcerated tissues according to Akinpelu and Onakoya [34].

Phytochemical screening of different solvent extracts of N. sativa showed various phytochemicals. The antibacterial activity of the selected seed extracts of *N. sativa* may be due to the presence of various phytochemicals seen in them. Similar observations were recorded by Cowan [35] and Padayana *et al.* [36] in which they have reported that antibacterial activity of leaf extracts can be attributed due to the presence of the phytochemicals. The results of the present study suggested that the use of this plant is beneficial to treat human diseases as it is a potential source of bioactive substances.

According to the results obtained, the presence of chemical constituents in the seeds of *N. sativa* supports its antimicrobial activity. These phytochemicals are known to show medicinal as well as physiological activity [37]. The occurrence of alkaloids, phenols, phytosterols, saponins, sterols, tannins, flavonoids, terpenoids in the aqueous root extracts of *N. sativa* was also reported earlier by Ali and Blunden [38]. Results parallel to present study was given by Srivastava *et al.* [39] who has reported the presence of flavonoid, tannin, steroid and triterpenes, saponins, alkaloids, cardiac glycosides and reducing compounds in the extract of *N. sativa.* Phytochemicals defined in the strictest sense, as chemicals produced by plants. However, the term is generally used to describe chemicals from plants that may enhance health status of organisms, but are not essential nutrients.

5. Conclusion

The medicinal value of plants depends on some chemical substances that produce some activities against various microbial infections. Plants produce a variety of biological compounds to protect themselves against a variety of pathogenic infections. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. The result suggested that the plant could be used as a curative agent for different ailments. In addition, phytochemicals evaluation of seed of *N. sativa* provided information about a number of medicinally important secondary metabolites, which impart antibacterial characteristics. It may be concluded from this study that the seed extract of *N. sativa* has antimicrobial activity against *S. aureus* and *S. typhimurium*. It is suggested that using natural products as an alternatives will probably not cause any health issues and instead of using synthetic drugs, plant sources can be used as a valuable therapeutic index. In conclusion, the results of this study showed that seed extracts of *N. sativa* have antibacterial activity against the most common bacterial strains *S. aureus* and *S. typhimurium* involved in human infectious diseases.

6. Financial Support and Sponsorship

This research did not receive any specific grant from funding agencies in the public and commercial sectors.

7. Conflict of Interest

There is no conflict of interest among the contributors

8. Reference

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