

ANTIBACTERIAL ACTIVITY OF *NIGELLA SATIVA* AND *PIPER NIGRUM*

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ABSTRACT

The aim of the present research was to find out the antibacterial activity of *Nigella sativa* and *Piper nigrum* against the 2 gram positive and 3 gram negative bacteria. The present research was carried out in the Microbiology Research Laboratory Hazara University Mansehra Khyber Pukhtunkhwa Pakistan during 17 January to 22 April 2011. In the present research Disc diffusion and Agar well plate method were used. The results of the Ager well plate method as more clear as compare to Disc diffusion method. The cold water, hot water and methanolic extract of the *Nigella sativa* have high activity against *Escherichia coli* 23mm, 22mm and 23mm respectively. The extracts of the said plant have shown low activity 18mm against *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The methanolic extract of the *Nigella sativa* have no activity against *Staphylococcus aureus*. The same extracts were also obtained from *Piper nigrum*. The cold water extract give good result 23mm against *Escherichia coli* while less effective against *Pseudomonas aeruginosa* 15mm. The cold water and methanolic extract have high activity against *Salmonella typhae*, *Escherichia coli* and low activity against *Pseudomonas aeruginosa* and *Enterococcus faecalis* respectively. Similarly the methanolic extract of *Piper nigrum* has shown no activity against *Staphylococcus aureus*. From the present research it was concluded that all the extract of *Nigella sativa* and *Piper nigrum* have high activity against gram negative bacteria as compare to gram positive bacteria.

Keywords: Antibacterial activity, Disc diffusion method, Agar well plate method, *Nigella sativa*, *Piper nigrum*

INTRODUCTION

Throughout the human history the medicinal plants were used for health care and still continue to make important contributions to health care (Mulliken, 2000; Schippmann *et al.*, 2002). For the treatment of different diseases the ancient civilization Greek, Roman, Chinese and Egyptian were used the medicinal plants (Aftab and Sial, 1999). According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases (Diallo *et al.*, 1999). The medicinal plants are important source of mineral, vitamin and trace element and regarded as a potentially safe drug (Pardo-de-Santayana *et al.*, 2007). The Pakistan is among those countries where the large populations use the medicinal plants for the health care. The Unani medicine system was brought by the Muslims scholars in to Indo– Pak subcontinent and practiced it for the country (Goodman *et al.*, 1995).

The traditional (plant-based) therapies are among the most frequently used in numerous parts of the world. The 75% population of French, Canada 70%, Australia 48%, USA 42%, and 38% population of Belgium use traditional medicine at least once in their life (WHO, 2002).

There has been a considerable interest to use plants and spices for the elimination of microorganisms because of increasing antibiotic resistance of microorganisms. Many plant derived products such as spices, fruit preparations, vegetable preparations or extracts have been used for centuries for the preservation and extension of the shelf life of foods (Chattopadhyay and Bhattacharyya, 2007). Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Spices include leaves (coriander, mint), flower (clove), bulbs (garlic, onion), fruits (red chilli, black pepper), stem (cinnamon), rhizomes (ginger, turmeric), seeds (kalonji) and other plant parts (Shelef, 1983). Prevention of pathogenic and spoilage microorganisms in food is usually achieved by using chemical preservatives but they are responsible for many carcinogenic and teratogenic attributes as well as residual toxicity and with growing concern of microbial resistance towards conventional preservatives, consumers tend to be suspicious of chemical additives and thus the exploration of naturally occurring antimicrobial for food preservations receives increasing attention (Ali *et al.*, 2007).

Nigella sativa seeds famous as black seed or black cumin which is belonging to the Family *Ranunculaceae*. The plants of *Nigella sativa* present in the Asia and Europe. Its seeds have an enormous medicinal significance and have been reported to exhibit many pharmacological properties that comprise antimicrobial activity against Bacteria, Fungi, Viral, and Parasites (Ali and Blunden, 2003). The seeds have also been used to treat bacterial, fungal and parasitic infections. The oil of the *Nigella sativa* is used for many diseases and also use as food preservative, condiment, carminative, analgesic in every parts of world (Salem, 2005). The seeds of *Nigella sativa* are composed of more than 100 compounds. A combination of volatile oils, fatty acids, flavonoids, saponins, proteins, and trace elements are thought to contribute to its value (Salihet *et al.*, 2009). Hazrat Abu Hurairah states-“*I have heard from Rasool Allah (P.B.U.H) that there is cure for every disease in Black seeds except death and Black seeds are shoonez*”(SahihBukhari).

Piper nigrum (black pepper) used as a traditional medicine. There are many compounds are present in the *Piper nigrum* but the most important and will known of these are piperidine and pyrrolidinealkamides. The piperine has many biological characteristics such as analgesic, stimulant of central nerves system, antipyretic and antifeedant activities. *Piper nigrum* is used as a natural spice. It is extensively cultivated all over the world and used as a spice. *Piper nigrum* have high function in antibacterial measures (Das *et al.*, 1998; Kirtikar and Basu, 1981; Linday, 1962; VenkatReddy *et al.*, 2004).

OBJECTIVE

The objective of the present research was to evaluate the antibacterial activity of *Nigella sativa* and *Piper nigrum* against some of the gram positive and gram negative bacteria.

MATERIALS AND METHODS

The present research was conducted in the Microbiology Research Laboratory of Hazara University Mansehra, Khyber Pakhtunkhwa, Pakistan. The present was carried during 17 January to 22 April 2011. So the brief account of materials as well as procedures used was described below.

Chemicals and Apparatus Used

Chemicals: Methanol, distilled water and media (MHA).

Apparatus: Centrifuge, Autoclave, Rotary Shaker, Micropipette, Test tubes, Beakers, Petri plates, Erlenmeyer flasks, Graduated Cylinders, Spirit lamp, Microwave oven, Incubator, Refrigerator, wire loop.

Plant Materials

Plant species *Nigella sativa* (kalonji) and *Piper nigrum* (black pepper) were purchased in dried form from the local market of Mansehra.

Preparation of Extracts from Nigella sativa and Piper nigrum

In order to obtain the plants extract 100gm of each dried plant (*Nigella sativa* and *Piper nigrum*) seeds or fruits were coarsely powdered with the help of pestle and mortar. Then the extracts were prepared with solvents like water and methanol by shaking the powder plants for 24 hours at 150rpm and the extract of different concentrations were prepared and then these extracts were used as antibacterial agents.

Cold water extraction of Nigella sativa and Piper nigrum

Take 4 Erlenmeyer flasks and in each flask take the 100ml of distilled cold water. Then addition of 10grams of each powdered plants (*Nigella sativa* and *Piper nigrum*) and soaked into the 100ml of distilled cold water and rotated on a rotary shaker at 150rpm for 24 hours. Then after 24 hours the extracts were filtered through filter paper and then centrifuged at 4500rpm for 5 minutes. After the centrifugation filtration through muslin cloth, pellet was discarded, supernatants, centrifuge again to make 100% pure extract.

Hot water extraction of Nigella sativa and Piper nigrum

Take the 4 Erlenmeyer flasks then take 100ml of distilled water in each flask then addition of 10grams of each powdered plants and soaked into 100ml of distilled water. Then the flasks were plugged and take over low flame of heat for 15 minutes. Then flasks were removed from heat and allow to cool and after cooling the contents of flask were filtered through filter paper and pure extract were obtain.

Methanolic extraction of Nigella sativa and Piper nigrum

Take four Erlenmeyer flasks then add 100ml of methanol then addition of powdered plants of 10grams in the 100ml of methanol. Then flasks were plugged with cotton and take on the shaker for shaking at 150rpm for 24 hours. After this the extracts were filtered through filter paper and centrifuged at 4500rpm for 5 minutes, then filtration, superintends again and again to make pure extract. All extracts were collected in Erlenmeyer flasks and covered with cotton or aluminum coil.

Test Bacteria

The antibacterial activity was determined against gram positive and gram negative microorganisms. The gram positive organisms include *Staphylococcus aureus* and *Enterococcus faecalis*. The gram negative bacteria include *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhae*.

Media Used for Culturing

Culturing media used for anti-bacterial assay was Muller Hinton Agar for the growth of respective bacteria.

Table 1. Composition of Mueller Hinton Agar

<i>S.No</i>	<i>Ingredients</i>	<i>Amounts</i>
1	Beef extracts powder	2.0 g/l
2	Acid Digest of Casein	17.5 g/l
3	Starch	1.5 g/l
4	Agar	1.7 g/l

Preparation of Media

Mueller Hinton Agar media was prepared in flask by dissolving 38gm of powdered agar media in 1 liter of distilled water. Then flask was shaken for some time to dissolve the medium completely and then sterilized the medium in an Autoclave at 121°C temperature for 15 minutes.

Methods Used for Applying Plant Extract***Disc Diffusion Method***

To determine the anti microbial activity of extracts make the discs from the blank filter paper with the help of punch machine and its size is 6mm. The extracts were taken in separate flasks and these disks were placed in these extracts to absorb adequate quantity for 3-5 minutes and then used against the bacterial species on the culture medium.

Agar Well Plate Method

It is another method applying the extracts on the culture medium. In this method the wells size of 0.5mm are made through sterile borer and then 30 to 60 ul of extracts was pour with the help of micro pipette.

Antibacterial Activity

Antibacterial activity was determined by the above two methods. For this purpose 10 ml of sterilized media was poured into the sterilized 15 Petri plates. Bacterial cultures were seeded on agar media, spreading for uniform growth over the entire surface of the media. First using the antibiotic disks on each plate and then the plates were incubated at 37°C for 24 hours. It gave inhibition zones but not completely clear so used the second method called Agar well plate technique and in this the same procedure followed and thus it gave a clear inhibition zone around the well, after the incubation of 24 hrs.

RESULTS

In the present study the antibacterial activity of cold water, hot water and methanolic extracts of *Nigella sativa* and *Piper nigrum* were checked against the gram positive bacteria *Staphylococcus aureus*, *Enterococcus faecalis* and gram negative bacteria *Escherichia coli*, *Salmonella typhae* and *Pseudomonas aeruginosa*. The result of the Ager well plate is more clear that of Disc diffusion method.

Antibacterial Activity of Cold Water Extract of *Nigella sativa* (seeds)

The cold water extract of *Nigella sativa* showed maximum zone of inhibition 23mm against *Escherichia coli*, followed by *Salmonella typhae* 21mm, *Staphylococcus aureus* 21mm, *Enterococcus faecalis* 20mm and *Pseudomonas aeruginosa* 18mm as shown in table 2.

Table 2. Zone of inhibition (mm) of cold water extracts of *Nigella sativa* seeds

Plant Extract	<i>Escherichia coli</i>	<i>Salmonella typhae</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Pseudomonas aeruginosa</i>
Cold water	23mm	21mm	21mm	20mm	18mm

Antibacterial Activity of Hot Water Extract of *Nigella sativa* (seeds)

Hot water extract showed maximum zone of inhibition of 22mm against *Escherichia coli*, *Salmonella typhae* and *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* 20mm and *Enterococcus faecalis* 18mm as shown in table 3.

Table 3. Zone of inhibition (mm) of hot water extracts of *Nigella sativa* seeds

Plant Extract	<i>Escherichia coli</i>	<i>Salmonella typhae</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>
Hot water	22mm	22mm	22mm	20mm	18mm

Antibacterial Activity of Methanolic Extract of *Nigella sativa* (seeds)

The methanolic extract showed maximum zone of inhibition 23mm against *Escherichia coli*, 20mm against *Salmonella typhae* and *Pseudomonas aeruginosa*, 18mm against *Enterococcus faecalis*, while no effect against the *Staphylococcus aureus* as shown in table 4.

Table 4. Zone of inhibition (mm) of methanolic extract of *Nigella sativa* seeds

Plant Extract	<i>Escherichia coli</i>	<i>Salmonella typhae</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>
Methanolic	23mm	20mm	20mm	18mm	Nil

Antibacterial Activity of Cold Water Extract of *Piper nigrum* (Fruit, Seed)

Cold water extract showed maximum zone of inhibition of 23mm against *Escherichia coli*, 21mm against *Enterococcus faecalis*, 20mm against *Staphylococcus aureus* and *Salmonella typhae* and 15mm against *Pseudomonas aeruginosa* as shown in table 5.

Table 5. Zone of inhibition (mm) of cold water extract of *Piper nigrum* fruits and seeds

Plant Extract	<i>Escherichia coli</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhae</i>	<i>Pseudomonas aeruginosa</i>
Cold water	23mm	21mm	20mm	20mm	15mm

Antibacterial activity of hot water extract of *Piper nigrum* (Fruit, Seed)

Hot water extract showed maximum zone of inhibition of 22mm against *Salmonella typhae* and *Staphylococcus aureus* followed by *Escherichia coli* 21mm, *Enterococcus faecalis* 19mm and *Pseudomonas aeruginosa* 18 mm as shown in table 6.

Table 6. Zone of inhibition (mm) of hot water extract of *Piper nigrum* fruits and seeds

Plant Extract	<i>Salmonella typhae</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Enterococcus faecalis</i>	<i>Pseudomonas aeruginosa</i>
Hot water	22mm	22mm	21mm	19mm	18mm

Antibacterial Activity of Methanolic Extract of *Piper nigrum* (Fruit, Seed)

The methanolic extract showed maximum zone of inhibition of 21mm against *Escherichia coli*, *Salmonella typhae* and *Pseudomonasaeruginosa*, 20mm against *Enterococcus faecalis* while no effect against the *Staphylococcus aureus* as shown in table 7.

Table 7. Zone of inhibition (mm) of methanolic extract of *Piper nigrum* fruits and seeds

Plant Extract	<i>Escherichia coli</i>	<i>Salmonella typhae</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>
Methanolic	21mm	21mm	21mm	20 mm	Null

DISCUSSIONS

In the present research the antibacterial activity of two medicinal plants *Nigella sativa* and *Piper nigrum* were checked against gram positive bacteria *Staphylococcus aureus*, *Enterococcus faecalis* and gram negative bacteria *Escherichia coli*, *Salmonellatyphae* and *Pseudomonas aeruginosa*. The cold water, hot water and methanolic extracts of these plants were obtained from the seeds and fruits. The cold water extract of *Nigella sativa* have high activity against *Escherichia coli* while less effective against *Pseudomonas aeruginosa*. Hot water extract shows high zone of inhibition against *Escherichia coli* and low against *Enterococcus faecalis*. The methanolic extract of *Nigella sativa* gives good result against the *Escherichia coli* and less effective against the *Enterococcus faecalis*. While the methanolic extract shows no activity against *Staphylococcus aureus*.

Similarly the cold water extract of the *Piper nigrum* shows high zone of inhibition against *Escherichia coli* and low against *Pseudomonas aeruginosa*. The hot water extract give good result against *Salmonellatyphae* and low against *Pseudomonas aeruginosa*. The methanolic extract shows high activity against *Escherichia coli*, *Salmonellatyphae* and *Pseudomonas aeruginosa* while low activity against *Enterococcus faecalis*. No activity has been reported against *Staphylococcus aureus*.

CONCLUSIONS

The overall results of the present research shows that the cold water, hot water and methanolic extracts of *Nigella sativa* and *Piper nigrum* have high zone of inhibition against gram negative bacteria (*Escherichia coli*, *Salmonella typhae* and *Pseudomonas aeruginosa*) as compare to gram positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*). The methanolic extract of both plants shows no activity against *Staphylococcus aureus*.

COMPETING INTEREST

The author declares that they have no competing interest.

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REFERENCES

- [1] Aftab, K. & Sial, A. A. (1999). Phytomedicine: New and old approach. *Hamdard Medicus*, 42(2), 11-15.
- [2] Ali, B. H. & Blunden, G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res.* 17, 299–305.
- [3] Ali, M. A., Alam, N. M., Yeasmin, M. S., Khan, A. M. M. & Sayeed, A. (2007). Antimicrobial screening of different extracts of *Piper longum* Linn. *Res. J. Agri. Biol. Sci.*, 3, 852-857.
- [4] Salih, B., Sipahi, T., Donmez, E. O. (2009). Ancient *Nigella* seeds from BoyaliHoyuk in North-Central Turkey. *J. Ethnopharmacol*, 124(3), 416-420.
- [5] Chattopadhyay, R. R. & Bhattacharyya S. K. (2007). Herbal spices as alternative antimicrobial food preservatives: An update. *Pharmacognosy Review*, 1(2), 239-247.
- [6] Das, B., Kasinatham, A. & Madhusudhan, P. (1998). “One new and two rare alkaloids from the two samples of *Piper longum*. *Natural Product Sciences*, 1(4), 23–25.
- [7] Diallo, D., Hveem, B., Mahmoud, M. A., Betge, G., Paulsen, B. S. and Maiga, A. (1999). “An ethnobotanical survey of herbal drugs of Gourma district”. *Pharmaceutical Biology*, 37(1), 80–91.
- [8] Goodman, S. M. & A. Ghafoor, (1995). *The Ethnobotany of Southern Balochistan, Pakistan, with particular reference to medicinal plants*. Chicago, Ill: Field Museum of Natural History, c1992. I-V, 1-84.
- [9] Kirtikar, K. R. & Basu, B. D. (1981). “*Indian Medicinal Plants*”. 2133.
- [10] Linday, M. E. (1962). *Practical Introduction to Microbiology*. 177.
- [11] Mulliken, T. (2000). Sustainable use of medicinal plants – A multi-sectoral challenge and opportunity. Harvesting of non-wood forest products. *Seminar Proceedings*, 2-8 October, Izmir.
- [12] Pardo-de-Santayana, M., Tard, O. J., Blanco, E., Carvalho, A. M., Lastra, J. J., San, M. E. & Morales, R. (2007). Traditional knowledge of wild edible plants used in the northwest of the Iberian Peninsula (Spain and Portugal): a comparative study. *J Ethnobiol Ethnomed*, 3(27). doi:10.1186/1746-4269-3-27
- [13] Salem, M. L. (2005). Immunomodulatory and therapeutic properties of *Nigella sativa* L. Seed. *International Immunopharmacology*, 5(13-14), 1749–1770.
- [14] Schippmann, U., Leaman, J. D. & Cunningham, A. B. (2002). *Impact of cultivation and gathering of medicinal plants on biodiversity: Global trends and issues*, Inter-Departmental Working Group on Biological Diversity for Food and Agriculture, Rome.
- [15] Shelef, L. A. (1983). “Antimicrobial effects of spices”. *Journal of Food Safety*, 6(1), 29-44.
- [16] Reddy, S., et al. (2004). “Antibacterial constituents from the berries of *Piper nigrum*.” *Phytomedicine*, 11(7-8), 697–700.
- [17] World Health Organization (WHO). (2002). WHO traditional medicine strategy 2002–2005. World Health Organization, Geneva, Switzerland.