Pak. J. Biochem. Mol. Biol. 2015; 48(4): 92-96

Evaluation of the antimicrobial activity of aqueous, ethanolic and methanolic extracts of *Nigella sativa* (kalonji) against gram positive and gram negative bacterial isolates

*Ujala Nasir and Saba Irshad**
Institute of Biochemistry and Biotechnology, University of the Punjab Lahore, Pakistan

Abstract: *Nigella sativa* (kalonji) has been used since ancient times as a nutritional supplement and for the treatment of various infections and chronic ailments. As the pathogens are becoming resistant to most of the drugs, kalonji can be used as an alternative compound to be used in modern medicines. The antimicrobial activity of kalonji was determined through disc diffusion and agar well diffusion method. All types of extracts of kalonji showed antimicrobial activity against all bacterial isolates with different zones of inhibition. In case of disc diffusion method, methanolic extract showed maximum antimicrobial activity against *B. subtilis*, *B. cereus* and *Corynebacterium sp*. Ethanolic extract showed highest antimicrobial activity against *E. coli*. Both ethanolic and methanolic extracts represented maximum antimicrobial activity for *Enterococcus sp*. In case of agar well diffusion method, ethanolic extract showed maximum antimicrobial activity against all of the bacteria except *E. coli*, where methanolic extract exhibited the maximum activity. Aqueous extract gave the least activity against all bacterial strains through both methods except for *S. aureus* by disc diffusion method where it exhibited the maximum antimicrobial activity. The statistical analysis showed the agar well diffusion method (M=17.46±2.02mm) and methanolic extract (M=16.08±3.61mm) to be most effective against bacteria. The aqueous extract (M=12.75±2.90mm) showed least activity. The *Enterococcus sp*. (M=16.75±3.50mm) was found to be most sensitive and *S. aureus* (M=12.75±6.04mm) was found to be least sensitive to the extracts of kalonji. Phytochemical screening showed the presence of flavonoids, tannins, saponins, alkaloids and steroids depicting the antimicrobial and antioxidant properties of kalonji.

Keywords: *Nigella sativa*, extracts, agar disc diffusion method, agar well diffusion method, antimicrobial activity, phytochemical screening.
Received: August 12, 2015 **Accepted:** November 5, 2015
***Author for Correspondence:** saba.ibb@pu.edu.pk

INTRODUCTION

Medicinal plants have been utilized for the health care throughout the human history. Still they are making significant contributions to health care[1,2]. These medicinal plants contain a wide variety of vitamins, minerals and trace elements which make them a very good alternative to synthetic drugs that are considered as unsafe for human as well as for the environment[3].

The *Nigella sativa* (Kalonji) seeds belong to Ranunculaceae family. The oil of kalonji is used as a condiment, analgesic, diaphoretic, liver tonic, stomachic, carminative and food preservative throughout the world. More than a hundred valuable constituents are found in its seeds which include fatty acids, volatile oils, proteins, carbohydrates, saponins, alkaloids, tannins, flavonoids, sterols and trace elements[4]. The seeds are used as diuretic, stimulant, astringent and anthelmintic. They are also helpful in preventing mouth's odor and watering. The decoction is helpful in the ailments like jaundice, piles, skin diseases and intermittent fever[5-8]. The mixture of kalonji and butter milk provides a solution for hiccups and vomiting. Different mixtures of kalonji are helpful in the treatment of obesity. If kalonji seeds are regularly consumed after frying, the conditions like catarrh and cold can be prevented. It is also effective in preventing migraine and persistent headache[9]. The seeds are effective for leprosy as well[10]. Hand and feet bulges can be treated by water solution of kalonji. The external uses of kalonji need the conditions like alopecia, freckles, eczema, pimples and leucoderma[9]. The active constituents found in kalonji are thymoquinone, nigellone and beta-sisterol. Thymoquinone is an active pharmacological component of the volatile oil which is found to be responsible for the improvement of respiration and decrease in the cholesterol, glucose serum levels and blood pressure[11,12]. It contains anti-inflammatory, pain killer and anti-oxidant impacts[13,14,5]. It is also responsible to fight human breast and ovarian cancer by arresting the cell cycle[15]. The *Nigella sativa* seeds are powdered and their extracts are made using different solvents. The solvents include methanol, ethanol, water, chloroform, n-hexane etc. The extracts of kalonji are quite helpful in curing non-insulin dependent diabetes mellitus[16]. The aqueous and methanolic extracts of kalonji along with hydrogen per oxide are responsible to make MCF-7 cell lines of breast cancer ineffective[17]. The volatile oil of black cumin seeds was prepared and *in vivo* study was carried out with an animal model. It showed the successful removal of local infection caused by *S. aureus*[18]. The antimicrobial activities of thymoquinone were determined against different microbial strains. Among sixteen oral strains, seven strains including *S. aureus*, *S. mutans*, *S. salivarius* and among eleven

laboratory reference strains, four strains including *S. epidermidis, B. cereus, M. luteus* and *S. aureus* were found to be sensitive to thymoquinone showing MBC and MIC values which ranged between 9 and 65 µg per ml. Four reference and six clinical bacteria were found inactive to thymoquinone having MIC ranges from 130 to 514 µg per ml. Moreover, the most resistant bacterium was found to be *P. aeroginosa* which showed Minimum Inhibitory Concentration of value greater than 514 µg per ml[19].

The ultimate objective of the current research was to determine the anti-microbial activity of *Nigella sativa* against six different types of bacteria i.e., *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Bacillus cereus, Corynebacterium* and *Enterococcus sp*. Agar disc and well diffusion methods were performed in this regard and their relative efficiency was also determined. The ability of this spice to fight against bacteria, thus preventing the disease, was monitored in this way. Three types of extracts of *Nigella sativa* were prepared in order to check the relative efficiency of the extracts causing inhibition in bacterial growth. The relative ability of the solvents was also tested to consider the most effective solvent in which the spice powder is efficiently dissolved and is allowed to fight against different types of bacteria. Moreover, the phytochemicals present within the spice were also evaluated in order to ensure the basic mechanism relating to the anti-microbial activity of kalonji.

MATERIALS AND METHODS

Collection and storage of Nigella sativa seeds

The seeds of *Nigella sativa* for the determination of antimicrobial activity were collected from a local market of Lahore. They were grinded with the help of clean pestle and mortar and were packed in polythene bags for further extractions. Then they were stored in a dry place in the research laboratory of Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore.

Preparation of Nigella sativa extracts

Three extracts of *N. sativa* were made in methanol, ethanol and distilled water. A 50 ml falcon tube was taken and 10 ml of solvent was added in it. Then 1 g of the powdered *N. sativa* was soaked in 10 ml of the solvent. Then it was rotated on the shaking incubator at 150 rpm for 24 h. Then after 24 h, the extract was filtered by filter paper and centrifuged at 3500 rpm for 15 minutes at 4ºC. After the centrifugation, the extract was filtered through muslin cloth. The pellet was discarded and the supernatant was centrifuged again and again until the extract made was 100 % pure. Then the extract was stored at 4ºC for future use. All of the extracts were prepared in similar ways.

Preparation of LB agar media

The culture plates were prepared for bacterial growth and antimicrobial effect determination. These petri plates were added with the LB agar medium which provided a solid support for the growth of bacteria and the visual determination of antimicrobial activity of *N. sativa*. The LB agar medium was prepared by adding 0.5 g of yeast extract, 1 g of trypton and 1 g of NaCl in 20 ml of distilled water. After dissolving the ingredients in distilled water, pH was adjusted to 7.4 with the help of pH meter. The distilled water was added to make the volume up to 100 ml. Then 1.5 g of agar was added at the end and stirred. Then it was autoclaved at 120ºC for 20 min.

Agar disc diffusion method to evaluate the antimicrobial activity

For performing disc diffusion method, discs were prepared from Whatmann No.1 filter paper that were having 6mm diameter. Then they were autoclaved at 120ºC for 20 min for sterilization. The solidified Luria Broth Agar media was allowed to melt on a hot plate. The media was then poured in sterilized petri plate. After the solidification of agar in the plate, 100 ul of the bacteria were allowed to spread on the nutrient agar petri plate by using sterilized spreader. After 10 min, forceps were taken with which that disc was hold and dipped in the extract of *Nigella sativa* for 10 sec. Then it was placed in the center of sterile nutrient agar plate. As a positive control, one disc was dipped in the working solution of ampicillin and placed in another plate in similar ways. As a negative control, one disc was dipped in the solvent alone (without kalonji) and placed in another plate in similar ways. The plates were then allowed to incubate for 24 h at 37ºC in the incubator. After 24 h, with the help of scale, zones of inhibition were determined in mm. All of the above steps were carried out in Laminar Flow Hood. The zones of inhibition were determined for all of the three extracts of *Nigella sativa* against all of the six bacteria in this manner.

Agar well diffusion method to evaluate the antimicrobial activity

The Luria Broth Agar was melted on flame and poured in petri plate. After solidification of agar in plate, 100 ul of the bacterial stock solution was allowed to spread in the nutrient agar plate using sanitized spreader. After a wait of 10 min, a sterilized cork borer was taken and with its help, a well was made in the center of the plate. It was filled with 40 ul of the extract. It was then placed in the

incubator for a day at 37ºC. A well was made in another plate which was filled with 40 ul of ampicillin as a positive control and incubated in similar ways. A well was made in another plate which was filled with 40 ul of the solvent only (without kalonji) as a negative control and incubated in similar ways. After 24 h, with the help of scale; the inhibition zone was determined in mm. All of the above steps were carried out in Laminar Flow Hood. The zones of inhibition were determined for all of the three extracts of *Nigella sativa* against all of the six bacteria in this manner.

Test for flavonoids

NaOH test was carried out to evaluate the existence of flavonoids in the aqueous extract of *Nigella sativa*. 10 % NaOH solution was made by dissolving 1 g of NaOH in 10 ml of distilled water. 5 ml of the aqueous extract of black cumin was taken in a test tube. Then 2 drops of 10 % NaOH solution were added to the test tube. The reaction was noted. To confirm the presence of flavonoids, 2 drops of 20 % HCl was added to the test tube. The color of the solution was noted.

Test for alkaloids

Mayer's reagent was used to determine the presence of alkaloids in the ethanolic extract of *Nigella sativa*. To make the Mayer's reagent, 1.36 g of mercuric chloride and 5 g of potassium iodide were added to 100 ml of distilled water in a beaker. Then 5 ml of the ethanolic extract of *Nigella sativa* was added in a test tube. 2 drops of the Mayer's reagent was added to the test tube. The reaction was noted.

Test for tannins

$FeCl_3$ test was used to check the presence of tannins in the water extract of kalonji. To perform the test, 5 ml of the boiled aqueous extract of *N. sativa* was added in a test tube. 2 drops of 5 % $FeCl_3$ was added to the test tube. The reaction was noted.

Test for steroids

H_2SO_4 test was used to test the presence of steroids in the methanolic extract of *Nigella sativa*. 2 ml of the methanol extract of kalonji seeds was added in a test tube. 2 ml of chloroform was put in the test tube. Then it was evaporated to dryness. Then a concentrated H_2SO_4 was allowed to enter by test tube's one side in order to form a lower layer. The reaction was noted.

Test for saponins

A distilled water test was used to find the presence of saponins in kalonji's aqueous extract. 3 ml of the boiled and filtered *Nigella sativa* extract was added in test tube. Then 3 ml of the distilled water was put in the tube. The solution was agitated for 5 min. After the shaking of 5 min, the reaction was noted.

Test for phlobatannins

HCl test was carried out to find the existence of phlobatannins in aqueous extract of black cumin. 5 ml boiled aqueous extract of kalonji was added in test tube. Then 5 ml 1 % HCl was allowed to enter the test tube. The solution was allowed to boil for 5 min. After boiling, the reaction was noted.

RESULTS AND DISCUSSION

The present research was conducted in order to ensure the antimicrobial activity of *Nigella sativa* by making its methanolic, ethanolic and aqueous extracts against one gram negative and five gram positive bacteria by using agar disc and agar well diffusion methods. All of the six bacteria showed different sensitivity for the kalonji extracts. This factor depends upon the ability of the particular bacteria to fight with the extracts of kalonji. The bacteria having more ability to fight with the extracts showed less zone of inhibition. Whereas, the bacteria having less or no ability to fight against the extracts showed high or maximum zone of inhibition. In a previous research, among the methanolic, ethanolic, n-hexane and aqueous extracts of kalonji, methanolic extract showed highest antifungal and antibacterial activity against all of the test fungi and bacteria respectively by agar well diffusion method[20].

In the case of *Bacillus subtilis*, maximum zone of inhibition of 19mm was shown by ethanolic extract of kalonji by agar well diffusion method. The methanolic and aqueous extracts were also good at their activities showing respective inhibition zones of 18mm and 17mm. whereas, in the previous research, methanolic extract showed maximum zone of inhibition of 24mm. The ethanolic and aqueous extracts showed 22 and 18mm respectively in agar well diffusion method[20]. In the present case, with disc diffusion method, methanolic extract represented maximum zone of 17mm. As the negative control (ethanol alone) showed 12mm zone of inhibition, so ethanolic extract showing 20mm zone of inhibition will not be considered to give maximum zone of inhibition. In the case of *Escherichia coli*, ethanolic extract showed the maximum zone of inhibition of 17mm by disc diffusion method which made it the efficient method, as compared to the agar well diffusion method through which methanol extract gave 16mm as the highest zone of inhibition. Agar well diffusion method appeared to be more efficient in giving the

highest inhibition zone i.e., 19mm in case of *Staphylococcus aureus* by ethanolic extract of kalonji. As the negative controls of methanol and ethanol also showed 15mm and 13mm zone of inhibition respectively by disc diffusion method, the methanolic and ethanolic extracts giving 21 and 16mm inhibition zones respectively will not be considered the true values. Whereas in the previous paper, it has been stated that methanolic extract showed no activity in both methods[21]. Maximum zone of inhibition of 20 mm was shown by ethanolic extract of kalonji against *B.cereus* by agar well diffusion method. Disc diffusion method did not appear to be efficient in giving the zone of inhibition as compared to agar well diffusion method i.e., the ethanolic extract showed only 12 mm zone of inhibition using disc diffusion method. Ethanolic extract of kalonji gave maximum zone of inhibition of 20 mm in the case of *Corynebacterium sp*. by agar well diffusion method. It showed similar zone of inhibition to ampicillin, which also gave 20 mm zone of inhibition. The methanolic extract also showed good antimicrobial activity, showing zone of inhibition of 18 mm. The methanolic extract of kalonji in case of disc diffusion method also showed zone of inhibition of 18 mm, which was highest in this case. In the case of *Enterococcus sp.*, both methanolic and ethanolic extracts of kalonji showed maximum 20 mm zones in the case of disc diffusion method. The results were similar to previous research work where both of the extracts gave 20 mm zones using the similar method[22]. The aqueous extract showed the zone of inhibition of 10mm only in the present case. While in agar well diffusion method, ethanolic extract showed highest zone of inhibition of 20 mm. the methanolic extract also showed good antimicrobial activity giving the zone of inhibition of 18mm.

Phytochemical screening was also done to determine the components present within *Nigella sativa*, as the basis of the antimicrobial activity of *Nigella sativa*. The flavonoids, alkaloids, tannins, steroids, saponins and phlobatannins were evaluated in the extracts of *Nigella sativa*. The flavonoids, alkaloids, tannins, steroids and saponins appeared to be present in the extracts, whereas, the phlobatannins appeared to be absent in the extract of kalonji. Yellow coloration indicated the presence of flavonoids and the solution became colorless on the addition of HCl, which confirmed the presence of flavonoids. Cream colored product appeared in the case of alkaloids, which ensured their presence. In the case of tannins, greenish precipitate appeared which indicated their presence. For steroids test, formation of reddish brown ring at the interface of two liquids confirmed their presence. Whereas for saponins, persistent froth was observed which indicated their presence. Moreover the absence of phlobatannins was observed in the extract of *Nigella sativa* as it remained colorless instead of giving red colored precipitate. But the results showed contradiction with the results shown previously, where the phytochemical constituents were evaluated for *Nigella sativa* which had steroids, tannins and flavonoids but lack saponins[23]. All of the phytochemicals were visually identified. The reason for the absence of phlobatannins might be due to inappropriate extraction of kalonji where the phlobatannins became missing in the extract. The presence of the phytochemicals ensured the ability of the *Nigella sativa* to have antimicrobial, antioxidant, antitumor and many other properties which can help to fight various diseases.

The statistical analysis was also performed in order to determine the most efficient method and extract against bacterial isolates. Moreover, the sensitivity of bacteria was also determined. The average zone of inhibition was determined in case of methods, bacteria and extracts independently. In case of methods, the average zone of inhibition was found M=13.1667, S.D=4.38046 in case of agar disc diffusion method. While for agar well diffusion method, the average zone of inhibition was obtained M=17.4583, S.D=2.02117 which was higher than the agar disc diffusion method. So the agar well diffusion method was found to be more efficient than the agar disc diffusion method (Table 1). In case of bacteria, the maximum average zone of inhibition was found M=16.7500, S.D=3.49489 for *Enterococcus sp*. Whereas for *Staphylococcus aureus*, the minimum average zone of inhibition was obtained M=12.7500, S.D=6.04152 which showed the highest sensitivity of *Enterococcus sp*. and least sensitivity of *Staphylococcus aureus* (Table 2). While comparing different extracts, the minimum average zone of inhibition was found M=12.7500, S.D=2.89592 for aqueous extract. Whereas in the methanolic extract, the maximum average zone of inhibition was obtained M=16.0833, S.D=3.60450 which was close to that of the standard drug (Ampicillin). So the methanolic extract was found to be most effective against the bacteria and aqueous extract was least effective (Table 3). The t-test was performed with methods and the t value (4.358, df=46) was found to be significant at level of significance. It also showed the higher efficiency of agar well diffusion method over agar disc diffusion method. The ANOVA was also performed with methods, bacteria and extracts independently. The results were significant for the comparison of two

methods but they were not significant in case of different bacterial strains and extracts as there was no significant difference of zones of inhibition among all of the bacteria and extracts.

CONCLUSION

The methanolic extract of *N. sativa* and agar well diffusion method have been found to show maximum antimicrobial activity. So the kalonji has the potential to inhibit the growth of various bacterial strains, thereby showing the ability of this spice to be used as food preservative. The active constituents of kalonji could be extracted and further used to make medicines for curing various diseases naturally.

Table 1: Average Zone of Inhibition (mm) by Agar Disc and Agar Well Diffusion Methods.

Methods	Mean	N	Std. Deviation	Median
Agar Disc Diffusion	13.1667	24	4.38046	13.5000
Agar Well Diffusion	17.4583	24	2.02117	18.0000
Total	15.3125	48	4.01145	16.0000

Table 2: Average Zone of Inhibition (mm) by *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Corynebacterium sp*. and *Enterococcus sp*.

Bacterial Isolates	Mean	N	Std. Deviation	Median
Bacillus subtilis	15.2500	8	4.33425	17.0000
Escherichia coli	15.1250	8	2.35660	15.0000
Staphylococcus aureus	12.7500	8	6.04152	14.5000
Bacillus cereus	15.7500	8	3.41216	17.0000
Corynebacterium sp.	16.2500	8	3.49489	17.0000
Enterococcus sp.	16.7500	8	3.49489	17.0000
Total	15.3125	48	4.01145	16.0000

Table 3: Average Zone of Inhibition (mm) by methanolic, ethanolic and aqueous extracts of *Nigella sativa*.

Type of Extracts	Mean	N	Std. Deviation	Median
Methanolic	16.0833	12	3.60450	17.5000
Ethanolic	15.5000	12	5.55141	18.0000
Aqueous	12.7500	12	2.89592	12.5000
Ampicillin	16.9167	12	2.42930	17.5000
Total	15.3125	48	4.01145	16.0000

REFERENCES

1. Mulliken T. Sustainable use of medicinal plants – A multi-sectoral challenge and opportunity. Harvesting of non-wood forest products. Seminar Proceedings, 2-8 October, Izmir, 2000.
2. Schippmann U, Leaman, JD and Cunningham AB. 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, 2002.
3. Pardo-de-Santayana M, Tard OJ, Blanco E, Carvalho AM, Lastra JJ, San ME and Morales R. Traditional knowledge of wild edible plants used in the northwest of the Iberian Peninsula (Spain and Portugal): a comparative study. *J Ethnobiol Ethnomed*. 2007; 3: 3-27.
4. Salih B, Sipahi T and Donmez EO. Ancient Nigella seeds from BoyaliHoyuk in North-Central Turkey. *J. Ethnopharmacol*. 2009; 124: 416-420.
5. Ali BH and Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother* Res. 2003; 17: 299–305.
6. Hosseinzadeh H, Bazzaz BSF and Haghi MM. Antibacterial activity of total extracts and essential oil of *Nigella sativa* L., seed in mice. *Pharmacol. Res.* 2007; 2: 429-435.
7. Paarakh MP. *Nigella sativa* Linn. *Ind. J. Nat Prod. Res*. 2010; 1: 409-429.
8. Gilani A, Aziz N, Khurram I, Chaudhary K and Iqbal A. Bronchodilator, spasmolytic and calcium antagonist activities of *Nigella sativa* seeds (Kalonji): a traditional herbal product with multiple medicinal uses. *J. Pak. Med. Assoc*. 2001; 51: 115-120.
9. Usmanghani K, Saeed A and Alam MT. Indusyunic Medicine: Traditional Medicine of Herbal, Animal and Mineral Origin in Pakistan. B.C.C. and T. Press, University of Karachi, Pakistan. 1997.
10. Treas and Evans. Pharmacognosy, international 15th edition 2002, saunder Edinburgh, New York: 258. 2002.
11. Al-Shabanah OA, Badary OA, Nagi MN, Al-garably NM, Al-Rikabi AC and Al-Bekairi A. Thymoquinone protects against doxorubicininduced cardiotoxicity without compromising its Antitumor activity. *J. Exp. Clin. Cancer Res*. 1998; 17: 193-198.
12. ElTahir KEH, Ashour MM and AlHarbi MM. The cardiovascular actions of the volatile oil of the black seed (Nigella sativa) in rats: Elucidation of the mechanism of action. *Gen. Pharmacol*. 1993; 24: 1123-1131.
13. Al-Gharably NM, Badry O and Nagi M. Protective effect of thymoquinone against CCl4-induced hepatotoxicity in mice. *Res. Comm. Pharmacol. Toxicol.* 1997; 2: 41-50.
14. Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA and Al- Behairy AM. Thymoquinone protects against CCl4- hepatotoxicity in mice via an antioxidant mechanism. *Biochem. Mol. Biol. Int*. 1999; 47: 153-159.
15. Shoieb AM, Elgayyar M, Dudrick PS, Bell KLand Tithof PK. *In vitro* inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. *Int. J. Oncol*. 2003; 22: 107-113.
16. Al-awadi F, Fatima H and Shamte U. The effect of a plant mixture extract on liver gluconeogenesis in Streptozoticin-induced diabetic rats. *Diabetes. Res*. 1991; 18: 163-168.
17. Farah IO and Begum RA. Effect of *Nigella sativa* and oxidative stress on the survival pattern of MCF-7 breast cancer cells. *Biomed. Sci. Instrum.* 2003; 39: 359-364.
18. Ara A. Study of the volatile oil of *Nigella sativa* against *Staphylococcus aureus*. *Microbiol. Rev.* 2015; 72: 101–123.
19. Kouidhi T. Determination of the antimicrobial activity of thymoquinone against sixteen oral strains. *Pak. J. Med. Sci*. 2011; 23: 37-42.
20. Ahmad R, Javed S, Shahid AA, Haider MS, Umeera A and Mushtaq S. Nutritional, phytochemical potential and pharmacological evaluation of *Nigella sativa* (Kalonji). *J. Med. Plants Res.* 2012; 6: 786-775.
21. Waqar M, Khan AU, Ali S, Rehman AU, Ali H, Ahmad T and Niaz Z. Antibacterial activity of *Nigella sativa*. *Asian J. Nat. Appl. Sci.* 2013; 2: 73-78.
22. Rafati J. Determination of the antimicrobial activity of *Nigella sativa* against pustules. *J. Ethnopharmacol*. 2014; 40: 3-9.
23. Salman KA. Antibacterial activity of *Nigella sativa* against multi drug resistant bacteria. *Microbiol. Rev.* 2010; 43: 83–90.