



Antifungal, Antibacterial, Phytotoxic and Insecticidal Activities of *Nigella sativa* Seeds

AMINA RAHAT¹, SHUMAILA BASHIR^{2*}, KHANZADI FATIMA KHATTAK³,
BASHIR AHMAD⁴, IBRAR KHAN⁴, SADIQ AZAM⁴, JAVID ALI⁵ and JAMSHAIH AHMAD⁴

¹College of Home Economics, University of Peshawar, Peshawar, Pakistan

²Department of Pharmacy, University of Peshawar, Peshawar, Pakistan

³Department of Chemistry, Abdul Wali Khan University, Mardan, Pakistan

⁴Center of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan

⁵PCSIR Laboratories Complex, Jamrud Road, Peshawar, Pakistan

*Corresponding author: Tel: +92 91 9216485; E-mail: saadb23@yahoo.com; saidhassan15@yahoo.com

Received: 14 April 2015;

Accepted: 20 May 2015;

Published online: 29 August 2015;

AJC-17514

The crude methanolic extract and different fractions (*n*-hexane, chloroform, ethyl acetate) of *Nigella sativa* seeds were screened for antifungal, antibacterial, phytotoxic and insecticidal activities. The methanolic extract showed total inhibitions against *Escherichia coli*, *Proteus mirabilis*, *Bacillus cereus* and *Xanthomonas oryzae* at 3 mg/mL. The *n*-hexane fraction showed significant antibacterial activity against *P. mirabilis*, *B. cereus* and *X. oryzae*, while CHCl₃ fraction had 100 % inhibition against *P. mirabilis*, *X. oryzae* and *E. coli*. Chloroform fraction possessed significant antifungal activity against *Penicillium oxalicum*, *Acremonium strictum*, *Verticillium lecanii* and *Trichoderma harzianum*. The methanolic extract exhibited significant activity (92 %) against *P. oxalicum*, good activity (64 %) against *A. strictum* and moderate activity (56 %, 42 %) against *V. lecanii* and *T. harzianum* respectively. The *n*-hexane, aqueous and EtOAc fractions were also found active against most of the test fungi. The methanolic extract showed good (75 %) phytotoxic activity against *Lemna minor*, while EtOAc and *n*-hexane fractions showed no phytotoxicity. Chloroform fraction showed 70 % mortality of *Tribolium castaneum*, while EtOAc and *n*-hexane fractions showed no activity against *T. castaneum*.

Keywords: *Nigella sativa*, Antibacterial, Antifungal, Phytotoxic, Insecticidal.

INTRODUCTION

Plants have been recognized for their therapeutic and medicinal potential since ages. A medicinal plant is one that contains substances which possess some therapeutic property or have the potential to be used as precursor for the synthesis of chemo-pharmaceutical products¹. Though plants are bestowed with large number of therapeutic chemicals but unfortunately most of these needs to be explored. Pakistan has a wide floral diversity consisting of about 6000 different genus and 700 species are being used for medicinal and aromatic purposes, but still the real commercial potential of these plants needs to be explored²⁻⁴.

The restorations of natural drugs started in last two decades mainly because of the broad and extended belief that green medicine is better than artificial products. Now-a-day, there are various medicinal plants based industries due to increase in the awareness of use of medicinal plants, all over the world, which are growing at a rate of 7-15 % yearly⁵.

About 60 % of the world's populations depend on the use of traditional plant extracts for their major health-care needs⁶.

At present, there are growing resistance against existing antibiotics and therefore a number of infectious diseases cannot be cured effectively^{7,8}.

World Health Organization (WHO) reported that about 80 % of the world population relies on the traditional medicine for the treatment of different diseases. Since 1980, WHO has been encouraging countries to identify and develop traditional medicine. Therefore, the assessment of rich heritage traditional medicine is indispensable.

In this regard, one such plant is *N. sativa* (Ranunculaceae), which is a small herb distributed and cultivated all over the world and regarded as one of the best healing medicine available. In Arabic known as *habbat al barakah* and commonly called "black seed" black cumin" or "fennel flower" in English, *N. sativa* is a very old food and medicinal harvest^{9,10}.

Our Prophet Muhammad (PBUH) told that the *N. sativa* has the healing power except death. Avicenna said that *N. sativa* is Canon of Medicine, restore the energy and recover from fatigue. It is found in the "Tibb-e-Nabavi and also in the Unani Tibb for its benefits^{5,11}.

The seeds of *N. sativa* have been used in pharmacological investigations. The studies have showed a broad spectrum of actions^{10,12,13}; the plant also showed antitumor activity¹⁴. The seeds of *N. sativa* are used for buffalo reproductive efficiency and for the treatment of *Helicobacter pylori* disease in human¹⁵⁻¹⁷. The seeds are used as diuretic, jaundice, intermittent fever, dyspepsia, paralysis, skin diseases and pile⁵. Seed oil is used as condiment, carminative, food additive and painkiller in different parts of the world^{18,19}. The world's biggest food company, Nestle is in search of rights on the use of *N. sativa* to stop food allergies⁹. Therefore keeping in view the importance of seeds of *N. sativa* the current study was aimed at screening it for antifungal, antibacterial, phytotoxic and insecticidal activities.

EXPERIMENTAL

The seeds of *N. sativa* were purchased from the local herbal market in Peshawar and identified by Prof. Dr. Farrukh Hussain, Department of Botany, University of Peshawar, Pakistan.

Extraction: Dried seeds of *N. sativa* (10 kg) were grinded to powder form and soaked in methanol for fifteen days at room temperature, with occasional shaking. The material was filtered and concentrated at 40 °C under vacuum by rotary evaporator. About 850 g of crude methanolic extract was obtained.

Fractionation: The crude methanolic extract of *N. sativa* (740 g) was dissolved in distilled water (500 mL) and partitioned with *n*-hexane (3 × 500 mL), CHCl₃ (3 × 500 mL) and EtOAc (3 × 500 mL). The fraction collected included *n*-hexane (150 g), CHCl₃ (155 g), EtOAc (130 g) and aqueous (200 g) fractions. 90 g of the methanolic extract was stored for biological and pharmacological activities.

Antibacterial activity: The methanolic extract and different fractions of *N. sativa* were tested for antibacterial activity against *E. coli*, *P. mirabilis*, *B. cereus* and *X. oryzae*. Stock solutions were prepared in dimethyl sulfoxide (DMSO, < 1 %) at concentration of 3 mg/mL of the test samples. The microorganisms were inoculated in nutrients broth (Sigma-Aldrich, Germany) and incubated at 37 °C. After incubation, 18-24 h culture of each test organism was transferred to sterile Nutrient agar plates for preparation of bacterial lawn and 6 mm wells were dug in plates at equal distance. 100 µL from the stock solutions of the test samples were loaded to their respective wells. For the positive and negative controls, amoxicillin and DMSO were used as controls respectively. The petri plates were left for 2-3 h undisturbed inside the laminar air flow to get the best diffusion of the test samples to media. The petri plates were then incubated at 37 °C for 24 h. The inhibition zones (mm) were calculated after incubation in comparison with positive control^{20,21}.

Phytotoxic bioassay: The test samples were checked against *Lemna minor* L. as per reported procedure²². Stock solutions of the test samples were prepared in methanol at concentration of 20 mg/mL and E-medium was prepared for the *L. minor* growth. 1000, 100 and 10 µg/mL from the stock solution were poured to three flasks, one for each sample and left at room temperature till the organic solvent was dried.

The E-medium and twenty healthy plants with a rosette of three fronds were added to all flasks and incubated at 28 ± 1 °C for seven days. Paraquat was used as standard growth inhibitor. Results were noted after seven days of incubation by counting the number of damaged plants^{21,22}.

Insecticidal activity: For this activity, direct contact toxicity was used²¹. Describing the procedure, the test sample (200 mg/mL) was dissolved in 3 mL of methanol and applied to filter papers (90 mm diameter). The dried filter paper was placed in the separate Petri dish along with 10 adults of *T. castaneum*. Permethrin (235.71 µg/cm²) was used as reference insecticide. These were kept without food for the interval of 24 h after which mortality was calculated.

Antifungal activity: Antifungal activity of the test samples were evaluated as per reported procedure²¹ against *P. oxalicum*, *A. strictum*, *V. lecanii* and *T. harzianum*. Sabouraud dextrose agar (Sigma-Aldrich, Germany) was prepared, autoclaved and 4 mL was dispensed into sterile test tubes. The (non-solidified) SDA media was mixed with test solution 66.6 µL. The tubes allowed for solidification in the slanted position at room temperature. An agar surface streak of the selected fungi was employed for non-mycelial growth. DMSO and standard antifungal drugs served as negative and positive control, respectively. After 7 days of incubation at 28 ± 1 °C linear inhibition was calculated.

Statistical analysis: In SPSS (two way anova) calculation showed highly significant difference between the different fractions (P = .000) and between the different concentrations 1000, 100 and 10 mg/mL it was insignificant (P = .076).

RESULTS AND DISCUSSION

Antibacterial activity: The demand of medicinal plants has increased with the advancement of science and increase in infectious diseases particularly caused by *E. coli*, *P. mirabilis*, *B. cereus* and *X. oryzae*, all becoming resistant to many antibiotics²³. This problem can be solved by the plant origin active ingredients; we have selected *N. sativa* which is locally available and traditionally used for different purposes. The previous research was conducted on different plant extracts e.g., *Zizyphus jujuba*²⁰, *Tylophora hirsute*²² and on *Myrsine africana*²¹ on different strains of bacteria^{10,13,24}. In our research methanolic extract possess significant antibacterial activity against *E. coli* (100 %), *P. mirabilis* (100 %), *B. cereus* (100 %) and *X. oryzae* (100 %). The *n*-hexane fraction possess significant activity against *P. mirabilis* (100 %), *B. cereus* (100 %) and *X. oryzae* (100 %) while low activity against *E. coli* (2 %). Chloroform fraction possess significant antibacterial activity against *P. mirabilis* (100 %), *X. oryzae* (100 %) and *E. coli* (100 %) while low activity against *B. cereus* (5 %). Aqueous fraction showed significant activity against *P. mirabilis* (100 %), *B. cereus* (100 %) and *X. oryzae* (100 %) while low activity against *E. coli* (30 %). The SPSS (two way anova) showed (P > 0.05) insignificant difference for different bacterial strains but significant difference for (P < 0.05) different fractions as shown in Fig. 1.

Antifungal activity: The methanolic extract of *N. sativa* showed significant activity against *P. oxalicum* (100 %), *A. strictum* (100 %), *V. lecanii* (100 %) and *T. harzianum* (100 %).

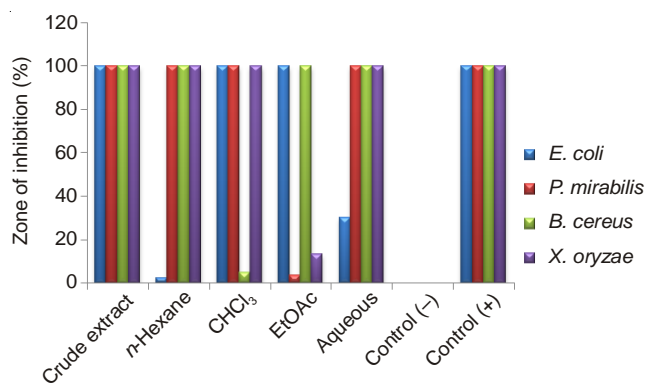


Fig. 1. Antibacterial activity of different fractions of *Nigella sativa* showing zone of inhibition (mm) Amoxicillin used as positive control

The chloroform fraction showed significant activity against *P. oxalicum*, (92 %) and good activity against *A. strictum* (64 %), moderate activity against *V. lecanii* (56 %) and *T. harizinum* (42 %). *n*-Hexane fraction showed good activity against *A. strictum* (69 %) and *T. harizinum* (61 %), moderate activity against *P. oxalicum*, (54 %) and *V. lecanii* (53 %). The EtOAc fraction showed significant activity against *A. strictum* (80 %) and good activity against *V. lecanii* (67 %) and *T. harizinum* (61 %), low activity against *P. oxalicum*, (26 %), aqueous fraction showed significant activity against *A. strictum* (86 %) good activity against *V. lecanii* (60 %) and moderate activity showed against *Trichoderma* (59 %) and low activity against *P. oxalicum* (19 %). The results of activity are given in Fig. 2.

The present research supports the work^{15,17} and expands the antifungal activity on other strains of pathogenic fungi.

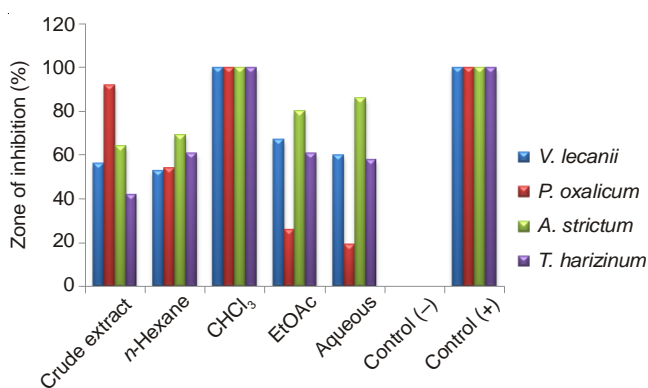


Fig. 2. Antifungal activity of different fractions of *Nigella sativa* showing percent linear inhibition (mm) miconazole used as positive control

Insecticidal activity: The plant origin insecticides are mostly environment friendly; therefore methanolic extract and various fractions of *N. sativa* were screened for insecticidal activity against *T. castaneum* using the contact toxicity assay²¹. The results are mentioned in Fig. 3; chloroform fraction showed good activity (70 %) against *T. castaneum* while low insecticidal activity (30 %) was shown by aqueous fraction and methanolic extract (20 %). EtOAc and *n*-hexane fractions showed no activity against *T. castaneum*. *N. sativa* showed significant difference in Pearson Chi-Square test = .000 (two-sided) between the fractions.

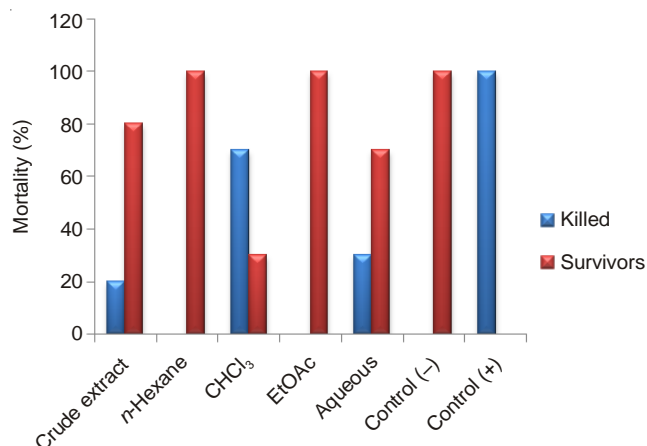


Fig. 3. Insecticidal activity of different fractions of *N. sativa* against the selected insects; Permethrin (235.9 mg/cm²) was used as standard drug

Phytotoxic activity: *L. minor* is small mono cotyledonous aquatic plant, sensitive to bioactive compounds. It has been used for the detection of natural antitumor, phytotoxic compounds and to identify new plant growth stimulant³. The methanolic extract showed good activity against *L. minor* (75 %) at 1000 µg/mL, moderate at 100 µg/mL (50 %) and low (40 %) activity at 10 µg/mL. The aqueous fraction showed good activity against *L. minor* (60 %) at 1000 µg/mL, moderate activity at 100 µg/mL (50 %) and low activity at 10 µg/mL (30 %). The chloroform fraction showed low activity at 1000 µg/mL (10 %) and 100 µg/mL (5 %). *n*-Hexane and EtOAc fractions showed no activity against *L. minor*. Results are shown in Fig. 4.

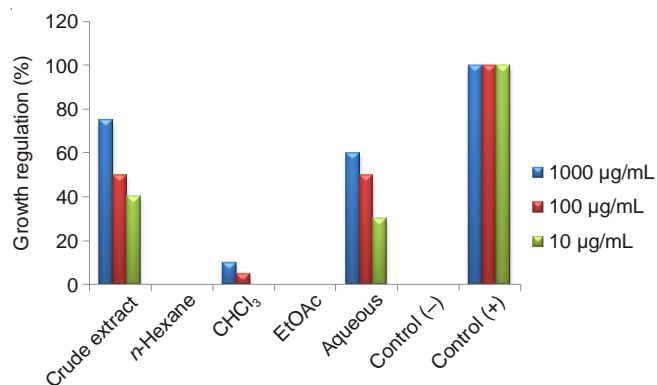


Fig. 4. Phytotoxic activity of the crude extract and fractions of *Nigella sativa* against *L. minor*; Paraquat (0.015 µg/mL) was used as standard drug

The results revealed that *N. sativa* seeds hold remarkable antimicrobial activity. Further research is in progress to isolate active compounds in this herb for manufacturing antimicrobial medicines.

REFERENCES

1. B. Patwardhan, A.D.B. Vaidya and M. Chorghade, *Curr. Sci.*, **86**, 789 (2004).
2. M. Ayesha, A. Parwez, V. Gupta, A. Mushtaq, U. Amin, S. Arfin and K. Janardhan, *J. Phcog.*, **2**, 351 (2010).
3. A. Rehman, *Studies in Natural Products of Chemistry*, **9**, 383 (1991).
4. A.Q. Rizwan, A. Mustaq and G.M. Asad, *J. Environ. Agric. Food Chem.*, **6**, 2500 (2007).

5. K.L. Chadha and M.L. Choudhary, Report of the Working Group on Horticulture, Plantation Crops and Organic Farming for the XI Five Year Plan (2007-12), Government of India, Planning Commission, p. 17 January (2007).
6. N.R. Farnsworth, Ethnopharmacology and Drug Development, Ciba Foundation Symposium 185, pp. 42-59 (1994).
7. A. Bashir, A. Sadiq and S. Bashir, *Afr. J. Biotechnol.*, **101**, 97 (2011).
8. A.R. McCutcheon, S.M. Elism, R.E. Hancock and G.H. Towers, *J. Ethnopharmacol.*, **37**, 212 (1992).
9. E. Hammond, Food Giant Nestlé Claims to have Invented Stomach Soothing use of Habbat Al-barakah (*Nigella sativa*), Nagoya Protocol ICNP-2, 2-6 July 2012, New Delhi (2012).
10. D. Zohary and M. Hopf, Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia, Europe and the Nile Valley, Oxford, p. 206 (2000).
11. H.G. Muhtasib, *J. Phytomed.*, **2**, 133 (2006).
12. B. Nickavar, F. Mojab, K. Javidnia and M.A.R. Amoli, *Z. Naturforschung*, **58c**, 629 (2003).
13. P.S. Rathee, S.H. Mishra and R. Kaushal, *J. Pharm. Sci.*, **44**, 8 (1982).
14. D.R. Worthen, O.A. Ghosheh and P.A. Crooks, *Anticancer Res.*, **18**, 1527 (1998).
15. A. Mohammad, *J. Ayub Med. Coll. Abbottabad*, **20**, 455 (2008).
16. E.M. Salem, T. Yar, A.O. Bamosa, A. Al-Quorain, M.I. Yasawy, R.M. Alsulaiman and M.A. Randhawa, *Saudi J. Gastroenterol.*, **16**, 207 (2010).
17. R. O'Mahony, H. Al-Khtheeri, D. Weerasekera, N. Fernando, D. Vaira, J. Holton and C. Basset, *World J. Gastroenterol.*, **11**, 7499 (2005).
18. K. Easterford, P. Clough, S. Comish, L. Lawton and S. Duncan, *J. Epilepsy Behav.*, **6**, 59 (2005).
19. M.L. Salem and M.S. Hossain, *Int. J. Immunopharmacol.*, **22**, 729 (2000).
20. B. Ahmad, I. Khan, S. Bashir, S. Azam and F. Hussain, *Afr. J. Biotechnol.*, **2011**, 2514 (2011).
21. B. Ahmad, S. Azam, S. Bashir, F. Hussain and M.I. Chaudhary, *Afr. J. Biotechnol.*, **108**, 1448 (2011).
22. B. Ahmad, N. Ali, S. Bashir and M.I. Choudhary, *Afr. J. Biotechnol.*, **8**, 4627 (2009).
23. G.A.J. Ayliffe, *J. Clin. Infec. Dis.*, **24**, 74 (1997).
24. A.J. Ferdous, S.N. Islam, M. Ahsan, C.M. Hasan and Z.U. Ahmed, *Phytother. Res.*, **6**, 137 (1992).