Phytochemical Activities of *Artemisia vulgaris* and *Acacia nilotica* Plant Extracts

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Authors’ contributions

This work was carried out in collaboration among all authors. Author SY designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FW and QA managed the analyses of the study. Author AM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

With the growing era, there is tremendous need to produce high potential antimicrobial drugs and medicines from various herbaceous plants and natural resources has been increasing due to increasing multiple drug resistance in pathogens. An experiment was performed to know about antimicrobial activities of various plant extracts. These activities were tested by using agar diffusion method. The bacterial as well as fungal strains were collected and were cultured on agar plates. After that these plates were left in incubator for 24 hours at 37°C to develop zones clearly all round the plant extracts. The activities of bacteria and fungi were determined by using inhibition diameter zones. The clear inhibition zones were found against bacterial strains in study. Antimicrobial activity of acetone, n-hexane and water extracts viz. *Acacia nilotica* and *Artemisia vulgaris* tested against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *E. coli* as well as *Aspergillus niger* and *Fusarium oxysporum*. Acetone extracts were found to be more effective as compared with n-hexane while water extracts show no activity.

Keywords: *Acacia nilotica*; *Artemisia vulgaris*; phytochemicals; antibacterial; antifungal.
1. INTRODUCTION

Since centuries, traditional medicinal plants have been used all over the international and they play a vital function in prevention and treatment of various illnesses. Medicinal herbs have always been famous in growing and used internationally due to their protection, efficacy, effortlessly availability and lesser side effects [1-3]. A lot of natural plants were used in conventional remedy as hepatoprotective from ages. *Acacia nilotica* (Mimosaceae) generally known as Babul. Acacia is the most common widespread genus of family *Leguminosae* firstly described by Linnaeus in 1773. The plant is a tress with yellow mimosa-like flowers and long grey pods constricted among seeds. The barks and branches bear spikes approximately 2 cm long. The leaves are 5 and densely bush with 3-6 pairs of pinnae including 10-20 pairs of leaflets that slender with parallel margins which are a rounded on the apex and with a central midrib intently crowded. The inflorescences encompass shiny yellow vegetation in auxiliary head on stalk. The flowering length of the plant is between November and March [4-6]. *Acacica nilotica* has been proved as powerful medicinal drug in treatment of malaria, sore throat (aerial component) and toothache (bark) [7]. The powdered bark of the plant with little salt is used for treating acute diarrhea [8-10]. Standardization is hard due to the fact natural drug are normally combination of components and the energetic essential in most cases is unknown. Therefore the prevailing have a look at changed into designed to standardized bark of *Acacia nilotica*.

*Artemissia vulgaris*, own family Compositae or Asteraceae usually known as Mugwort pollens (Hindi). Mugwort pollen is considered one of the major assets of hay fever and allergic bronchial asthma, in North Europe, North America and in components of Asia [11,12]. The fern like leaves of many species are covered with white hairs. The stem is thin woody and the plant produced in an annual fragrant north and south Indian flora. Mugwort pollen is an aggressive and invasive plant. Most of the species are determined growing wild and abundantly all over the temperate and cold temperate zones of the arena. It is barely poisonous and plenty of pharmacological sports of *Artemissia vulgaris* were reported: Prolonged dosage can damage the nervous system [13]. All parts of the plant are anthelmintic, antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, digestive, emmenagogue, expectorant, nervaine, purgative and stimulant. The leaves are harvested in August and can be dried for later use. The leaves are also stated to be appetizer, diuretic, haemostatic and stomachach [14-16]. An infusion of the leaves and flowering tops is used within the remedy of nervous and spasmodic affections, sterility, practical bleeding of the uterus, dysmenorrhoea, asthma and diseases of the brain [17,18]. The leaves have an antibacterial motion, inhibiting the growth of *staphylococcus aureus, Bacillus typhi, B. dysenteriae, streptococci, E. coli, B. subtilis, Pseudomonas*. The stem is also stated to be antirheumatic, antispasmodic and stomachach [14].

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant (*Artemissia vulgaris*) and (*Acacia nilotica*) were collected from local markets of city Lahore and were identified at the Institute of Molecular Biology Biotechnology, The University of Lahore. The collected material was included flowers, leaves, roots, shoots and pods then crushed in to fine powered form one by one using mortar and pestle.

2.2 Extraction

Six unique extracts have been prepared each from *Acacia nilotica* and *Artemissia vulgaris* by using three different solvents: acetone, n-hexane and water. 20 grams of each powdered sample had been one by one measured and soaked in 100 ml of solvents individually in 250ml of conical flasks and the flask mouth has been covered with the use of aluminum foil. The flasks were simultaneously stirred after same intervals so the both sample and solvent mix together well and after that the mixtures were left for 48 hours. After 48 hours, the supernatant was separated from the mixture and the ultimate extract to be filtered via filter paper.

2.3 Rotary Evaporation

The filtrate solution then undergoes for rotary evaporation. The rotary evaporator is the most preferred technique for solvent removal within laboratory. The filtrates had been heated in water bath at round 60 °C till the solvent evaporated to dryness for 12 hours. The remaining solidified extracts had been then collected in smooth
Eppendorf tubes. The tubes had been labeled for identity.

2.4 Storage and Protection of Extracts

The prepared extracts have been preserved in fridge till use. The bottles were Stoppard cautiously and labeled as it should be proceeding to storage at room temperature.

2.5 Maintenance and Conservation of Microbial Samples

The strains of micro organism and fungal used in this study were collected from Institute of Molecular Biology Biotechnology, The University of Lahore.

2.6 Antimicrobial Activity Test of Plant Extracts

Agar well diffusion technique is usually used for the primary screening of the antimicrobial activity of plant extracts towards particular pathogens in vitro.

2.7 Preparation of Nutrient Media

In order to make 380 ml of media, 57 grams of Agar and 7.6 grams of Nutrient broth must be used. Keeping this situation steady, the required volume of media had been prepared whenever. Using digital balance the powder turned into measured proceeding to consisting of distilled water. After stirring to interrupt off any lumps, the flasks were then heated on a Bunsen burner until the solution turned clean and bubbles appeared. After permitting to cool for a couple of minutes, the mouth of conical flask containing the media was covered using aluminum foil and then autoclaved. After sterilization, the media was cautiously pored at once to sterile petri dishes using laminar air flow cabinet. For medium- sized plates, the volume required is 20 ml and for massive plates volume required is 35 ml [19-21].

2.8 Inoculation and Agar Well Diffusion

The samples (Psuedomonas, Ecoli and Bascillius) and for fungal (Fusorium and Aspergillus) had streaked on freshly organized Nutrient agar plates. The streaking was done with the help of loop and Bunsen burner to remove any contamination. With the use of loop, each microbial strains had been cultured on freshly prepared media plates to obtain even growth. For inoculation of plant extracts, sterile cork borer was used into bore wells in agar plates. The extracts (100 µl) had been then loaded with inside the wells by the use of micropipette. The plates were then wrapped with parafilm and incubated at 37°C for 24 hours. 24 hours is an only estimated time, as bacteria usually takes approximately 1 hour to grow and fungus takes entire 24 hours to grow [19,21].

Anti bacterial and Anti fungal activities of the extracts were determined with the aid of using measuring the diameter of inhibition zones in millimeters produced in opposition to the pathogens. The experiments had been repeated 3 instances and the mean values had been calculated.

2.9 Preparation of 1M DPPH Solution

To prepare 1M DPPH solution, the 4 mg of DPPH has been taken in flask and 100 ml of 99% of pure methanol was added in it. DPPH must be measured before methanol has been added into it. Flask was heated continuously to mix the require sample well. Flask mouth has been covered tightly with aluminum foil and the prepared mixture was put under control conditions. Afterwards samples were put inside water bath at 100 rpm at room temperature [22].

2.10 Centrifugation

Following it samples were then taken from water bath tub and again undergo stirring for blending the solvents completely. Then the processes of centrifugation have been performed. Centrifugation is a way of retaining apart molecules having one in every type of densities via the use of spinning them in solution around an axis of centrifuge rotor at excessive velocity. After that unique time tubes had been removed from centrifuge and the supernatant was removed with the assist of filter paper. After that series of that particular solution has been made by way of using extracted solution and methanol of 1 ml, 2 ml, 3 ml, 4 ml and 5 ml. 1 ml of every sample has been taken an d3 ml of DPPH solution is poured in each pattern. After that each sample has set a part with 99% methanol as much as 10 ml. then the organized pattern was stored a side for about 30 minutes. After that, the samples are sooner or later passed via spectrophotometer assay.
3. RESULTS

3.1 Antibacterial Activity Test Results

Antibacterial and Antifungal activities of n-hexane, acetone and aqueous or water extracts from exclusive plants had been tested against bacterial strains consisting of *E. coli* and *Bacillus* samples. The results received are mentioned below (Tables 1 and 2).

3.2 Antifungal Activity Test Results

Antifungal activity of n-hexane and acetone extracts from various plants was tested towards two different fungal strains such as *Aspergillus niger* and *Fusarium oxysporum* samples. The results obtained from this study are mentioned below (Tables 3 and 4).

3.3 Antioxidant Activity Test Results

The results obtained from antioxidant tests are presented below (Tables 5 and 6), The higher antioxidant activity was found for samples of *Acacia nilotica*.

4. DISCUSSION

Acetone, n-hexane and aqueous extracts of different plants viz. *Acacia nilotica* and *Artemissia vulgaris* have proved to be effective against the human pathogens. Firstly there are three kinds of bacterial strains viz. *Psuedomonas aeruginosa*, *Bascillus subtilis* and *Escherichia coli* have been used. Those extracts show the exclusive sector if inhibition for the dose of 2.5 µl and 5 µl towards all the bacterial strains. Tables above illustrate the antimicrobial activities inhibited via acetone, n-hexane extracts of diverse plants towards several bacterial species respectively. For *Psuedomonas and Bacillus*, the best inhibition zones have been produced by means of acetone extracts towards strains and for *Escherichia coli* the very best inhibition zone regarded for n-hexane extracts towards sample. N-hexane and acetone extracts were extra effective than the ethanol extracts.
[23-25]. The most inhibition for the bacterial growth represents the extracts act as antibiotics and it could be used for controlling the growth of diseases, bacterial strains in the host cellular which help in preventing from several sicknesses by those bacterial strains [26-28]. For measuring the inhibition zones towards fungal species viz. *Aspergillus niger* and *Fusarium oxysporum* use. The different inhibition zones for the dose 2.5µl and 5µl have been taken against fungal strains. The tables mentioned above demonstrate all the antifungal activities shown by acetone and n-hexane extracts of various plants against the fungal strains respectively. For *Aspergillus niger* the most prominent zone of inhibition produced by acetone extracts against sample. The both n-hexane and acetone extracts were more effective than that of aqueous extracts [29-31]. Antioxidants are in particular essential materials which possess the capability to prevent diseases because free radical induced oxidative stress. The antioxidants against *Acacia nilotica* methanol extracts bring new studies inside field of new bioactive compounds from natural assets. It has been proven that *Acacia nilotica* as well as *Artemissia vulgaris* possess exceptional antioxidant activities in comparing to Vitamin C for DPPH scavenging interest [32,33]. Antioxidant activity was clearly determined in *Acacia nilotica* and somehow in *Artemissia vulgaris*. Methanolic extract of herbal plants *Acacia nilotica* and *Artemissia vulgaris* having different concentrations from 0-20 µl/mL were showed enormous absorbance of DPPH free radical i.e., for *Acacia nilotica* from 0.301-0.284 and inhibition percentage started from 5.86% to 65.82% respectively and for *Artemissia vulgaris* from 0.350-0.311 and percentage started from 7.42% - 57.0%. At the same concentration of aqueous extract of *Acacia nilotica*, [34] reported 59.80% scavenging of DPPH free radical [35-38].

Table 1. Antibacterial effect of inhibition zones (mm) produced by acetone, n-hexane extracts of *Acacia nilotica*

| Bacterial strains    | Dose 2.5 µl | Dose 5 µl | Control conditions |
|----------------------|-------------|-----------|--------------------|
| *Bacillus subtilis*  | 10          | 14        | 0                  |
| *Escherichia coli*   | 3           | 6         | 0                  |
| *Pseudomonas aeruginosa* | 8    | 11        | 0                  |

Table 2. Antibacterial activity of inhibition zones (mm) produced by acetone, n-hexane extracts of *Artemissia vulgaris*

| Bacterial strains    | Dose 2.5 µl | Dose 5 µl | Control condition |
|----------------------|-------------|-----------|-------------------|
| *Bacillus subtilis*  | 3           | 6         | 0                 |
| *Escherichia coli*   | 5           | 8         | 0                 |
| *Pseudomonas*        | 7           | 5         | 0                 |

Table 3. Antifungal effect inhibition zones (mm) produced by acetone and n-hexane extracts of *Acacia nilotica*

| Fungal strains       | Dose 2.5 µl | Dose 5 µl | Control conditions |
|----------------------|-------------|-----------|--------------------|
| *Aspergillus niger*  | 9           | 15        | 0                  |
| *Fusarium oxysporum* | 11          | 19        | 0                  |

Table 4. Antifungal effect inhibition zones (mm) produced by acetone, n-hexane extracts of *Artemissia vulgaris*

| Fungal strains       | Dose 2.5 µl | Dose 5 µl | Control conditions |
|----------------------|-------------|-----------|--------------------|
| *Aspergillus niger*  | 11          | 18        | 0                  |
| *Fusarium oxysporum* | 12          | 20        | 0                  |
Table 5. Antioxidant effect produced by methanol extracts of *Acacia nilotica*

| Obtain extracted solution (ml) | Concentration mg/ml | Absorbance (A) | Inhibition % |
|-------------------------------|---------------------|----------------|-------------|
| Control                       | 0                   | 0.301          |             |
| 1                             | 4                   | 0.284          | 5.86        |
| 2                             | 8                   | 0.271          | 10.07       |
| 3                             | 12                  | 0.249          | 17.26       |
| 4                             | 16                  | 0.203          | 32.52       |
| 5                             | 20                  | 0.103          | 65.82       |

Table 6. Antioxidant effect produced by methanol extracts of *Artemisia vulgaris*

| Obtain extracted solution (ml) | Concentration mg/ml | Absorbance (A) | Inhibition % |
|-------------------------------|---------------------|----------------|-------------|
| Control                       | 0                   | 0.350          |             |
| 1                             | 4                   | 0.311          | 7.42%       |
| 2                             | 8                   | 0.305          | 31.1%       |
| 3                             | 12                  | 0.299          | 39.9%       |
| 4                             | 16                  | 0.285          | 45.1%       |
| 5                             | 20                  | 0.270          | 57.0%       |

5. CONCLUSION

Organic solvent extracts of *Artemisia vulgaris* and *Acacia nilotica* have been powerful against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Fusarium oxysporum* isolates. Plant extracts inhibited bacterial growth correctly than the industrial antibiotics. The give up result of this investigation concluded that use of natural vegetation in treating microbial infections and the examine show that the plant life could be used as new antimicrobial agents. It can be hoped that studies like this could contribute to the set up such compounds that may be used to formulate antimicrobial of medicine of natural beginning. Furthermore research is wanted for the system standardization and optimization. Research is likewise needed to discover, isolate and purify the exact bioactive components which are probably chargeable for such antimicrobial homes of those plants.

CONSET

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lodhi M, Memon Z, Shaheen S, Wasim A. Effect of methanol extract of swertia chirata on various cellular components of blood in rats. International Journal of Medical Research and Health Sciences. 2017;6(8):59-64.
2. Ahmad S, Hera Z, Hanif M, Syed A. Effects of carbosulfan on the biology of bird cherry oat aphid. Biol Clin Sci Res J. 2020;e014.
3. Ali J AQ, Hafeez MM, Malik A. Clinical features, diagnosis and treatment of COVID-19. Biol Clin Sci Res J. 2020; e032.
4. Mohammed SF, Ibrahim IT, Saleh MS, Hassabelrasoul RM, Garbi MI, et al. Anti-bacterial activity of methanolic fruits extract of *Acacia nilotica* (L.). International Journal of Pharmaceutics and Pharmacology. 2017;1(4).
5. Ali Q, Khalil R, Nadeem M, Hafeez, MM, Malik, A. Antibacterial, antioxidant activities and association among plant growth related traits of Lepidium draba. Biol Clin Sci Res J. 2020;2020e011.
6. Balqees N, Ali Q, Malik A. Genetic evaluation for seedling traits of maize and wheat under biogas wastewater, sewage water and drought stress conditions. Biol Clin Sci Res J. 2020;e038.
7. Bargali K, Bargali S. Acacia nilotica: a multipurpose leguminous plant. Nature and Science. 2009;7(4):11-19.
1. Gill L. Ethnomedical uses of plants in Nigeria. Chapter: Book name of publication; Uniben Press; 1992.
2. Danish P, Ali QH, MM, Malik A. Antifungal and antibacterial activity of aloe vera plant extract. Biol Clin Sci Res J. 2020; e003.
3. Ejaz RM, S Ahmad, M Ali, H Choudhry, S. Anti-biofilm potential of menthol purified from Mentha piperita L. (Mint). Biol Clin Sci Res J. 2020; e037.
4. Li X, Hu J, Wang X, Zhang H, Liu J. Moxibustion and other acupuncture point stimulation methods to treat breech presentation: A systematic review of clinical trials. Chinese Medicine. 2009; 4(1):4.
5. Hameed B, Ali Q, Hafeez M, AM. Antibacterial and antifungal activity of fruit, seed and root extracts of Citrullus colocynthis plant. Biol Clin Sci Res J. 2020; e033.
6. Stuart M, Stuart M. The Encyclopedia of Herbs and Herbalism; 1979.
7. Duke JA, Ayensu ES. Medicinal plants of China. Chapter: Book name. 1985 of publication; 2; Reference Publications.
8. Kamran, Ali Q, Malik A. Genetic evaluation of legume species under heavy metal and biogas wastewater treatments. Biological and Clinical Sciences Research Journal. 2021; (1):e001.
9. Khalil R, Ali QHM, Malik A. Phytochemical activities of Conocarpus erectus: An overview. Biol Clin Sci Res J. 2020; 2020e008.
10. Gaddamwar A, Rajput P, Jirapure S. Manufacture of soft-plywood and hard-plywood from Acacia Nilotica Plant extract and powder of agricultural waste or wood. International Journal of Applied Engineering Research. 2011; 2(1): 78.
11. Mushtaq UM, S Afzal, M Ali, Q Malik, A. Role of modern technology for treatment of HCV. Biol Clin Sci Res J. 2020;2020e001.
12. Siddique A Fa, Idrees N, Hafez Mm, Ali Q, Malik A. The epidemics of COVID-19 BioClin Sci Res J. 2020;2020e030.
13. Ur Rahman A, Shakoor A, Zaib G, Mumtaz AS, Ihtesham Y, et al. Comparative antimicrobial activity of Acacia nilotica L. leaves extracts against pathogenic bacteria and fungi. Journal of Medicinal Plants Research, 2014;8(29):975-982.
14. Tabassum SA, Bibi T, Tariq F, Tariq S, Raza S, et al. Unusual leukemoid reaction in a COVID-19 patient: a case report. Biological and Clinical Sciences Research Journal. 2020;2020(1):e034.
15. Bilal M, Nasir I, Tabassum B, Akrem A, Ahmad A et al. Cytotoxicity and in-vitro antiviral activity of lectin from Crocus vernus L. against potato virus Y. Applied Ecology and Environmental Research, 2020;18(1):1301-1315.
16. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences. 2004,74(17):2157-2164.
associated with CLCuD. Biol Clin Sci Res J. 2020;2020e002.
34. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, et al. Oxidative stress, prooxidants and antioxidants: the interplay. BioMed Research International; 2014.
35. Bila, M, Nasir I, Tabassum B, Akrem A, Ahmad A, Ali, Q. Cytotoxicity and in-vitro antiviral activity of lectin from Crocus vernus l. against potato virus Y. Applied Ecology and Environmental Research. 2020;18:1301-1315.
36. Ahmad M, Ali Q, Hafeez M, Malik A. Improvement for biotic and abiotic stress tolerance in crop plants. Biological and Clinical Sciences Research Journal. 2021; 2021(1):e004.
37. Malik A, Hafeez K, Nazar W, Naeem, M., Ali I, Ali Q, Ahmed Mujtaba Z, Rana M Hafeez M. Assessment of controversial risk factors in development of breast cancer: a study from local population. Biological and Clinical Sciences Research Journal. 2021;(1):e003.
38. Khalid S, Ali Q, Hafeez M, Malik A. Perception regarding self-medication of antibiotics in general public sector university of southern punjab: a comparison between medical and non-medical students. Biological and Clinical Sciences Research Journal. 2021;(1): e005.

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