ABSTRACT

Spinach (Spinacia oleracea L. SO) leaves represent an important dietary source, have high nutritional value and antimicrobial properties. Spinach leaves have been used in the treatment of human diseases since ancient times. Here, the aim of this study was to evaluate the antimicrobial and antifungal activities of ethanolic extract of Spinach oleracea leaves by determining the minimum inhibitory concentrations (MICs) using well diffusion method against bacterial species Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus and fungal species Aspergillus fumigatus, Aspergillus niger.
**1. INTRODUCTION**

Our ecosystem, environment and human health is going to be destroyed due to the excessive use of antibiotics. All pathogenic bacteria have resistance factor against antimicrobial drugs therefore bacteria are major failure in the treatment of infectious diseases [1-5]. So, it is necessary to develop alternative solutions to control resistant bacteria. One of the possible strategies is phytochemicals that have antibacterial activity against pathogens. Researchers investigated that plants are major source of antimicrobial agents [6,7]. Many medicinal plants such as Coriander, parsley, oleander, myrtle, mint, henna, Aloe vera, christ’s thorn, olive, chamomile, cinnamon, licorice, and ginger are source of therapeutic agents [8,9]. Coriander (Coriandrum sativum) is a rich source of vitamins, nonanal, decanal, linalool and many useful substances. It has resistance against almost all Gram positive and negative bacteria. Nerium oleander has antimicrobial activity against Gram positive and Gram negative bacteria. Myrtus communis species are very rich source of volatile oils phenolic acids, flavonoids, fatty acids, anthocyanin pigments and tannins. Mentha piperita has effective antimicrobial properties against 21 pathogen microorganisms [10-13]. Henna’s bark, leaves, and seeds are rich source of phenolic compounds such as flavanol, Lawstone, mannitol, fat, resin, mucilage, tannin, gallic acid, glucose and phenolic acid that are used in medicine [4,14-18]. Aloe Vera leaves are a good source of great biologically active compounds, such as anethrones and various lectins. Aloe vera has antifungal, antiviral and antibacterial activity against skin infections. Spinach oleracea is one of them a good dietary source and nutritional value. Spinacia oleracea has good efficiency against bacterial and fungal species.

Researchers investigated that plants are major source of phenolic compounds such as flavanol, gallic acid, glucose and phenolic acid that are used in medicine [4,14-18]. Aloe Vera leaves are a good source of great biologically active compounds, such as anethrones and various lectins. Aloe vera has antifungal, antiviral and antibacterial activity against skin infections. Spinach oleracea is one of them a good dietary source and nutritional value. Spinacia oleracea is rich in amino acid, iron, vitamin K, vitamin A and folic acid. It contains almost all minerals, and is richest source of carotenoids, beta-carotene and lutein [19-21]. This is a good source of vitamin K (8) which helps in blood clotting. Spinach is beneficial source for various carotenoids and lipophilic active compounds and decreasing the risk of many diseases such as heart disease, diabetes, neurodegenerative disease and obesity [17,22-26]. The present study was conducted to evaluate antimicrobial effects of ethanolic extracts of Spinach oleracea root, stem and leaves against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella enteric isolates. These bacteria are resistant to the most common antibiotics.

2. MATERIALS AND METHODS

2.1 Extract Preparation

Root, stem and leaves of Spinach oleracea were collected from the Botanical garden of GCUL, Pakistan. All plant parts were dried under sunlight and converted into fine powder. About 60 grams of each sample in powdered form was added to 250 ml of ethanol as solvent and then left for a week and filtered using Whatman filter paper. The filtrate was dried by using rotary evaporator under reduced pressure at 50°C temperature. The crude extracts were weighed by using weighing balance and were dissolved into dimethyl sulfoxide or DMSO as a solvent to obtain required concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml.

2.2 Preparation of Inoculums

Inoculums were standardized to adjust the density of 10^6 CFU/ml. Five ml of nutrient broth with test organism was inoculated into it then incubated it at 3°C for 24 h. Take 0.2 ml of bacterial culture was dispensed into 20 ml of sterile nutrient broth and then was incubated for 3–5 h to attain the density 10^6 CFU/ml (corresponding to 0.5 McFarland standards). Plates were inoculated within 15 min to avoid changes in inoculum density.
2.3 Antimicrobial Assay

Well diffusion method was conducted to evaluate the antimicrobial activities. Muller Hinton agar plates were prepared for bacterial growth and Sabouraud dextrose agar plates were prepared for fungal growth. All petri plates were labeled for bacterial and fungal species. Five mm wells were cut and 10 μl of each concentration of plant extracts (stem, leaves and root extracts with different concentrations) were poured into wells. Positive and negative controls were put on the culture plates. DMSO was used as a negative control and Vancomycin was used as a positive control. All plates were incubated at 37°C for 22-24 hours. After the incubation period, diameter of the zones of inhibition was measured by using measuring scale.

Chart 1. Test microorganisms

| Gram positive | Gram Negative | Fungal Species |
|---------------|---------------|---------------|
| Staphylococcus aureus | Pseudomonas aeruginosa | Aspergillus fumigatus |
| Bacillus subtilis | Klebsiella pneumonia | Aspergillus niger |
| C. colocynthis | Fusarium oxysporum | Candida Albicans |

3. RESULTS

Table 1 showed that all bacterial and fungal strains were highly sensitive against the extracts of stem, leaves and root of *Spinach oleraceae*. The results indicated that leaf extract showed high activity against Gram-positive bacteria than Gram-negative bacteria. Maximum activity of *S. aureus* from 10 mm at 25 mg/ml to 21 mm at concentration of 100 mg/ml in leaf extract. Leaf extract showed highest zone of inhibition against *A. fumigatus* from 9 mm (at 25 mg/ml) to 18 mm (at 100 mg/ml). Roots showed highest zone of inhibition against bacteria *B. subtilis* 10 mm (at 25 mg/ml) and 22 mm (at 100 mg/ml ) while in the case of fungus root showed highest zone of inhibition against *A. fumigatus* 10 mm (at 25 mg/ml) and 20 mm (at 100 mg/ml). Stem extract showed highest zone of inhibition against bacteria *B. subtilis* 10 mm (at 25 mg/ml) and 21 mm (at 100 mg/ml) while fungus *F. oxysporum* is highly sensitive for stem extract, which showed highest zone of inhibition against 14 mm (at 25 mg/ml) and 20 mm (at 100 mg/1ml DMSO). Both the bacterial and fungal strains showed greater zones of inhibition by increasing the concentrations of the extracts.

4. DISCUSSION

The effect of *Spinach oleraceae* plant extracts was evaluated by using stem, leaves and roots at different concentrations 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml against the bacterial and fungal strains. All the organisms were highly sensitive against the extracts but the lowest the zone of inhibition was observed in stem extract as compare to others [27,28]. Gram-positive bacteria (*S. aureus* and *B. subtilis*) showed high zone of inhibition as compared to gram-negative bacteria and zone of inhibition were also increased by increasing the concentrations of the extracts. *C. colocynthis* has high antimicrobial property against gram negative bacteria *Pseudomonas aeruginosa*. All plant extracts showed active antifungal against all the fungal strains especially *C. albicans*. The efficiency of extracts activity was increased by increasing the concentration of the extracts. Activity of plant extract depends upon the plant organs and the nature of solvent [29].

![Fig. 1. Zone of inhibition of ethanolic root extracts against bacterial and fungal species](image-url)
Table 1. Antibacterial and antifungal activity of Spinach oleraceae root, stem, leaf extract against bacterial and fungal species

| Plant parts | Microorganisms              | Concentration (mg/ml) and zone of inhibition(mm) |
|-------------|-----------------------------|--------------------------------------------------|
| Root        | Bacterial species           | 25 50 75 100 + Control - Control                |
|             | Staphylococcus aureus       | 13 19 19 21 27 0                               |
|             | Bacillus subtilis           | 10 14 20 22 25 0                               |
|             | Pseudomonas aeruginosa      | 7 11 19 19 19 0                                |
|             | Klebselia pneumonia         | 12 14 17 20 19 0                               |
|             | Fungal Species              |                                                  |
|             | Aspergillus fumigatus       | 10 14 17 20 20 0                               |
|             | Aspergillus niger           | 6 10 18 19 19 0                                |
|             | Candida Albicans            | 8 13 16 18 20 0                                |
|             | Fusarium oxysporum          | 14 19 17 21 20 0                               |
| Stem        | Bacterial species           | 25 50 75 100 + Control - Control                |
|             | Staphylococcus aureus       | 9 10 13 14 19 0                                |
|             | Bacillus subtilis           | 10 7 19 21 19 0                                |
|             | Pseudomonas aeruginosa      | 8 12 13 16 6 0                                 |
|             | Klebselia pneumonia         | 10 12 14 19 20 0                               |
|             | Fungal Species              |                                                  |
|             | Aspergillus fumigatus       | 11 14 15 19 19 0                               |
|             | Aspergillus niger           | 13 16 16 21 20 0                               |
|             | Candida Albicans            | 11 11 13 18 18 0                               |
|             | Fusarium oxysporum          | 14 18 18 20 21 0                               |
| Leaves      | Bacterial species           | 25 50 75 100 + Control - Control                |
|             | Staphylococcus aureus       | 10 15 18 21 24 0                               |
|             | Bacillus subtilis           | 12 14 15 20 23 0                               |
|             | Pseudomonas aeruginosa      | 11 12 16 18 24 0                               |
|             | Klebselia pneumonia         | 14 16 14 18 27 0                               |
|             | Fungal Species              |                                                  |
|             | Aspergillus fumigatus       | 9 15 14 18 22 0                                |
|             | Aspergillus niger           | 11 14 21 22 21 0                               |
|             | Candida Albicans            | 9 12 14 17 20 0                                |
|             | Fusarium oxysporum          | 9 12 13 14 22 0                                |
5. CONCLUSION
The study concluded that ethanolic extracts of *Spinach oleracea* plant has a good efficiency against human pathogens. At low concentration 25 mg/1ml DMSO, zone of inhibition was very small that ranges from 6mm to 14mm but at high concentration 100 mg/1 ml DMSO, zone of inhibition was very large which ranges from 6mm to 27mm, which indicated that activity of plant extracts was increased by increasing the concentrations of the extracts. This study was conducted to evaluate the antifungal and antibacterial activity of different plant part (leaves, Stem and root) and concluded that leaf extract was very efficient against four bacterial and four fungal species and natural products can be a good source for the treatment of heart disease, diabetes, neurodegenerative disease and obesity.

CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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