Journal of Pharmaceutical Research International

33(22B): 94-100, 2021; Article no.JPRI.66985
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Antibacterial and Antifungal Activity of Plant Extracts from *Spinacia oleraceae L.* (Amaranthaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Author FS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UN, AZ, SA, TR, RS and MN managed the analyses of the study. Authors Q. Akram and Q. Ali managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i22B31410
Editor(s):
(1) Dr. Giulio Tarro, Foundation T. & L. de Beaumont Bonelli for cancer Research, Italy.
Reviewers:
(1) Farah Farhanah Haron, Malaysian Agricultural Research and Development Institute, Malaysia.
(2) Lucía Nitsch-Velásquez, Centro Universitario de Zacapa, Guatemala.
Complete Peer review History: http://www.sdiarticle4.com/review-history/66985

Original Research Article

Received 02 January 2021
Accepted 08 April 2021
Published 14 April 2021

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 *Klebseilla pneumonia and fungal species Aspergillus fumigatus, Aspergillus niger,*

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Candida albicans and *Fusarium oxysporum*. We evaluated from present data that ethanolic extract of root showed zone of inhibition ranges from 6 mm to 21 mm, ethanolic extract of stem showed zone of inhibition ranges from 8 mm to 21 mm and ethanolic extract of leaf showed zone of inhibition ranges from 9 mm to 22 mm from concentration 25 mg/ml to 100mg/ml. Leaf extract has high antibacterial and antifungal activity against bacterial and fungal species while root extract has low antibacterial and antifungal activity against bacterial and fungal species. Activity of plant extract was increased by the increasing concentration of extracts. Very low zone of inhibition was found at concentration 25 mg/1 ml DMSO which ranges from 6 mm to 14 mm while very high zone of inhibition was found at concentration 100 mg/ml which ranges from 6 mm to 22 mm. So, ethanolic extracts of *Spinach oleraceae* has good efficiency against bacterial and fungal species.

Keywords: Spinacia oleracea; antibacterial; antifungal; ethanolic extract; inhibition zones.

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, Lawsone, 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 anthrones 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 oleraceae* 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 from 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 in to 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℃ for 22-24 hours. After the incubation period, diameter of the zones of inhibition was measured by using measuring scale.

Chart 1. Test microorganisms

	Bcaterial species
Gram positive	*Staphylococcus aureus*
	Bacillus subtilis
Gram Negative	*Pseudomonas aeruginosa*
	Klebseilla pneumonia
	Fungal Species
	Aspergillus fumigatus
	Aspergillus niger
	Candida Albicans
	Fusarium oxysporum

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].

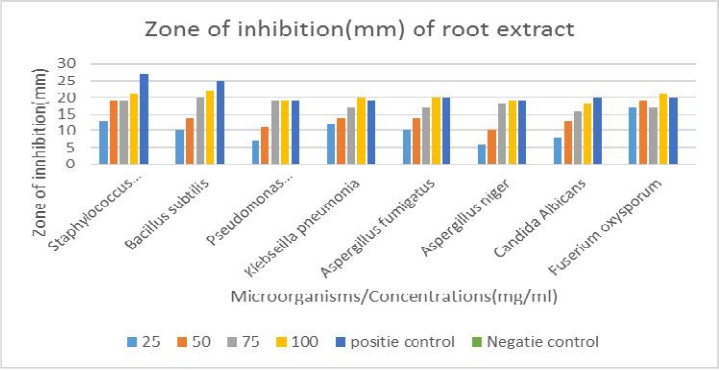

Fig. 1. Zone of inhibition of ethanolic root extracts against bacterial and fungal species

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)					
			25	50	75	100	+ Control	- Control
Root	Bacterial species	*Staphylococcus aureus*	13	19	19	21	27	0
		Bacillus subtilis	10	14	20	22	25	0
		Pseudomonas aeruginosa	7	11	19	19	19	0
		Klebseilla 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	*Staphylococcus aureus*	9	10	13	14	19	0
		Bacillus subtilis	10	7	19	21	19	0
		Pseudomonas aeruginosa	8	12	13	16	6	0
		Klebseilla 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	*Staphylococcus aureus*	10	15	18	21	24	0
		Bacillus subtilis	12	14	15	20	23	0
		Pseudomonas aeruginosa	11	12	16	18	24	0
		Klebseilla 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

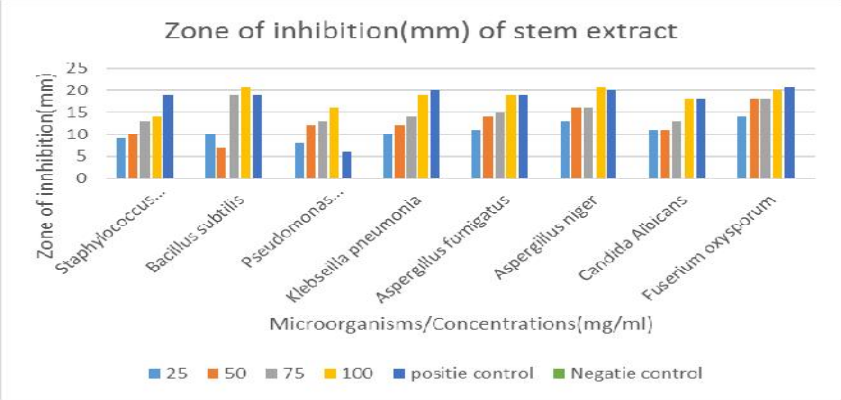

Fig. 2. Zone of inhibition of ethanolic stem extracts against bacterial and fungal species

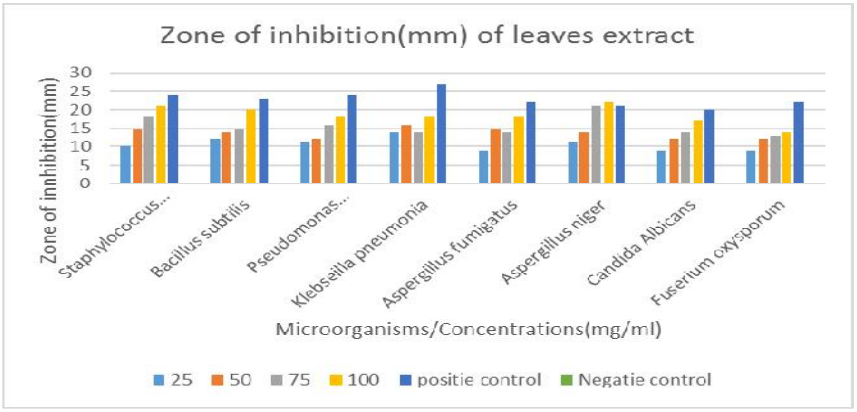

Fig. 3. Zone of inhibition of ethanolic leaves extracts against bacterial and fungal species

5. CONCLUSION

The study concluded that ethanolic extracts of *Spinach oleraceae* 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 antifugal 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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Peer-review history:
The peer review history for this paper can be accessed here:
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