



Antibacterial and Antifungal Activity of *Ficus carica* Plant Extract

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Authors' contributions

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

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ABSTRACT

The development of antibiotic resistant bacteria causes many harmful effects on human and animal lives. In developing countries many expensive synthetic drugs are being used to cure diseases but they have many side effects. Therefore, there is need to develop new strategies to control microbial infections. Therefore, we decided to work on extracts of different parts of *Ficus carica* which have good activity against gram positive bacteria, gram negative bacteria and fungal species. Methanol and chloroform extracts of root, stem, leaves and fruits were prepared and zone of inhibition was measured by using well diffusion method against gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) two gram positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*) and two fungal species *Aspergillus niger* and *Aspergillus oryzae*. methanol extract of leaves has high % yield (5.86%) and high zone of inhibition (23mm) against bacteria *Escherichia coli* and

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fungus *Aspergillus niger* (34mm). Bacteria *Staphylococcus aureus* was highly sensitive to chloroform extract with zone of inhibition 74mm while *Escherichia coli* was highly sensitive to metabolic extract with zone of inhibition 82mm. Chloroform extract has average zone of inhibition (56mm) and methanolic extract has average zone of inhibition (63mm).

Keywords: *Ficus carica*; *escherichia coli*; *aspergillus niger*; antibiotics; drugs; methanolic.

1. INTRODUCTION

Pharmacological industries have produced large number of antibiotics in the last three decades. But bacteria are genetically resistant to these drugs. Therefore large number of patients in hospitals has to face new infections due to high motility rate [1-4]. Therefore, it is necessary to reduce this problem. This problem can be overcome by develop other new drugs or develop new research to better understand the genetic mechanisms of resistance. Therefore use of plant compounds for pharmaceutical purposes has gradually increased. Medicinal plants are the best source to obtain a variety of drugs. Approximately 80% of individuals obtained medicines from compounds, derived from medicinal plants [2,5-7]. The plant extracts and phytochemicals both have antimicrobial properties and have great significance in therapeutic treatments. Plants have ability to produce a wide variety of secondary metabolites such as alkaloids, flavonoids, terpenoids, saponins, glycosides, steroids, tannins, quinones and coumarins. These biomolecules are the good source of plant-derived antimicrobial substances [8-11]. These natural products are highly efficient for bacterial treatment.

One of the surveys conducted by the World Health Organization (WHO) reports that 80% of the world's population depends upon the traditional medicines for various diseases. But there are numerous plants and natural products which have antibacterial, antifungal, and antiprotozoal activity that could be used for disease treatment [12-17]. Plants have been also medicinal properties throughout the world, due to their low toxicity, and economic viability as compared to synthetic drugs [18-23]. The antimicrobial activities of different plants have been investigated by a number of researchers worldwide. *Moringa oleifera* is one of the best known plant species belongs to family Moringaceae. The plant has antimicrobial properties and used in the treatment of human diseases. *Matricaria recutita* or *Matricaria chamomilla* commonly known as chamomile that belongs to family Asteraceae. It is widely used

and well-documented medicinal plants [24-26]. It has been used as a medicinal plant for external wounds, eczema, skin irritations, leg ulcers, inflammation of the skin, diaper rash, bacterial skin diseases, and many others. In Argentina, a researcher tested 122 known plant species and concluded that the compounds extracted from these plants inhibited bacterial growth, twelve inhibited the growth of *Staphylococcus aureus*, four inhibited *Aspergillus niger*, ten inhibited *Escherichia coli*, and also reported that the most potent compound was one extracted from *Tabebuia impetiginosa*. Many other studies have been conducted in Brazil [9,12,27]. The antimicrobial activity of *Vatairea macrocarpa* on *Klebsiella spp.* and *S. aureus* and the antifungal activity of extracts from *Eucalyptus spp.* was observed. A more detailed study was done on antimicrobial compounds by evaluating the extracts obtained from 120 plant species from 28 different families. It was documented that 81 extracts were obtained from 58 different plants that were active against *S. aureus*, and five extracts from four other plants inhibited the growth of *P. aeruginosa*. Another study was carried out to detect the antibacterial and antifungal (*C. albicans*) activity of essential oils obtained from *Croton triangularis* leaves [2,8,10,28,29]. Extracts from *Lippia gracilis* and *Xylopi sericea* showed antifungal activity. The investigation of antimicrobial activity as well as cell toxicity of extracts from 30 plant species against five bacteria species and two fungi species was studied. It was concluded that ethanol extracts from 70 % of the plants were toxic to cell and only one of the species of *Combretum duarteianum* showed antimicrobial activity [30-32]. The toxicity of extracts from *Arthemus sativa*, which is known to have antimicrobial activity, was also studied. The present study was carried out to estimate the antibacterial activity of aqueous, chloroform, and methanol extracts of *Ficus carica* leaves, roots, stem and fruit against gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, gram positive bacteria *Staphylococcus aureus*, *Bacillus cereus* and fungal species *Aspergillus niger* and *Aspergillus oryzae* [33,34].

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

The plant *Ficus carica* was collected and washed with tap water then dried different plant part root, stem, leaves, and fruit at room temperature for 20 days. This plant belongs to family Moraceae. Make a fine powder of each plant parts (Root, stem, leaves, fruit) separately by using pestle and mortar Table 1. Take 60g of fine root powder and dissolved it in 150ml methanol then left it for 8 days at room temperature. After 8 days filtrate it by using Whatman filter paper and residue was used for chloroform extraction. Same procedure was repeated for each plant part. After filtration filtrates were evaporated under reduced pressure at 50°C by using rotary evaporatory. Crude extract was collected for future investigation. The extract yields were was calculated and stored it in small flasks at 5 °C and their yield percentages were calculated using the formula:

$$\text{Extract yield\%} = \frac{\text{weight of extracted plants residues}}{\text{weight of plant raw sample}} \times 100$$

Extracts were analyzed by using TLC. Methanol extract was contained tannins, polyphenols, Xanthophylls, Flavanol, Terpenoids, Saponins, Anthocyanins, Tatarols, Phenones while chloroform extract was contained Terpenoides and Flavanoides.

2.2 Test Cultures

The culture bacterial strains were included, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* while fungus strains were *Aspergillus niger* and *Aspergillus oryzae*.

2.3 Preparation of Inoculum

A loopful of the microorganisms was inoculated into 5.0 ml of nutrient broth and incubated it at 37 °C for 24 h. Take 0.2 ml from this microbial culture and dispensed it into 20 ml sterile saline water then its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10⁷ CFU/ml (which was corresponding to 0.5 McFarland standards) by using spectrophotometer. Plates were inoculated within 15 min of standardizing the inoculum, to avoid any changes in density of inoculums.

2.4 Well Diffusion Method

Well diffusion method was applied for antibiotic sensitivity test against different bacterial species. Muller Hilton agar was poured into sterilized petriplates and left it for cool. Media become solidify. Take 100µl of inoculum and spreaded it on to Muller Hilton agar plate. Petriplates were labeled according to bacterial and fungal samples with black permanent marker. Four wells of 6-mm diameter were punched off into the agar medium with sterile cork-borer (6 mm) and filled them with 100 µl (500 mg/ml) of each plant extract by using a micropipette. DMSO was used as a negative control. The plates were incubated at 37 ± 2 °C for 24–48 h. The antibacterial screening was evaluated by measuring the zone of inhibition (mm).

3. RESULTS AND DISCUSSION

3.1 Plants Extraction Yield

The table illustrated that 1-4% yield was obtained from chloroform extract and 2-5% yield was obtained from methanolic extract of root, stem, leaves and fruit. The crude extract of all plant parts indicated that percentage yield is directly proportional to the polarity of solvents. Polarity indices of methanol were 5.1 and chloroform polarity indices was 4.1, moreover difference in % yield depends upon the solubility of different constituents of plant material in different solvents Table 2.

3.2 Zone of Inhibition

Antimicrobial activity of different plant parts were investigated against two strains of gram positive bacteria *Staphylococcus aureus*, *Bacillus cereus* and two strains of gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*. While antifungal activity of plant *Ficus carica* was tested against two fungal species *Aspergillus niger* and *Aspergillus oryzae*. Well diffusion method was adopted for this purpose. Zone of inhibition was measured by using measuring scale [10,22,35]. This ethanomial data in table indicated that Methanolic extract was high antimicrobial and antifungal activity as compare to chloroform extract because Methanolic extract has high % yield (2-5%) and high polarity indices (5.1). Average zone of inhibition of methanolic extract was 63mm Table 3, while average zone of inhibition of chloroform extract was 56mm. In methanolic and chloroform extracts, leaves

extract is highly effective as compare to others. *Staphylococcus aureus* was highly sensitive to chloroform extract of leaves (34mm). While *Aspergillus niger* was highly sensitive to methanolic extract of leaves (34mm). *S aureus* is one of the most important bacteria that cause human diseases like skin and soft tissue infections it can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections. *Aspergillus niger* also affect human health such as immune deficiency, fungus molds cause serious alveolitis allergies and asthma [3,9,12,36]. But the leaves of ficus carica have high antibacterial and anti-fungal activities against *Staphylococcus aureus* and *Aspergillus niger*. Chloroform extracts have high zone of inhibition against gram positive bacteria (74mm) while methanolic extracts have high zone of inhibition against gram negative bacteria (82mm). In the case of fungus Chloroform extracts have high zone of inhibition against *Aspergillus oryzae* (55mm) while methanolic extracts have high zone of inhibition against fungus *Aspergillus niger* (79mm).

Fig. 1 indicated the antimicrobial and antifungal activity of different chloroform extract of root, stem, leaves and fruit. The chloroform extracts of root showed high antimicrobial activity against

bacteria *Bacillus cereus* that showed 16mm zone of inhibition while lowest antimicrobial activity was observed against bacteria *Staphylococcus aureus* that showed 11mm zone of inhibition. In case of stem, stem showed high antimicrobial activity against bacteria *Bacillus cereus*, that showed 15 mm zone of inhibition [8,32]. High antimicrobial activity of leaves was observed against bacteria *Staphylococcus aureus* that showed 34 mm zone of inhibition. Fruit showed high antimicrobial activity against bacteria *Staphylococcus aureus* that showed 15mm zone of inhibition [37-39]. Antifungal activity of different plant parts were observed and illustrated that all plant parts showed high antifungal activity against fungal specie *Aspergillus orezae*.

Fig. 2 indicated the antimicrobial and antifungal activity of different methanolic extracts of root, stem, leaves and fruit. The methanolic extracts of root, stem, leaves and fruit showed high antimicrobial activity against bacteria *Escherichia coli*, while lowest antimicrobial activity was observed against bacteria *Pseudomonas aeruginosa*. Antifungal activity of different plant parts were observed and illustrated that all plant parts showed high antifungal activity against fungal specie *Aspergillus niger*.

Table 1. Quantity of plant material and solvents (ml) employed during investigation

Plant parts	Plant material(g)	Solvent(ml)
Root	60	150
Stem	150	400
Leaves	80	300
Fruit	15	75

Table 2. % extraction yield of different plant parts

Plant parts	% extraction yield	
	Chloroform	Methanol
Root	2.23	3.59
Stem	3.87	5.57
Leaves	4.02	5.86
Fruit	1.13	2.79

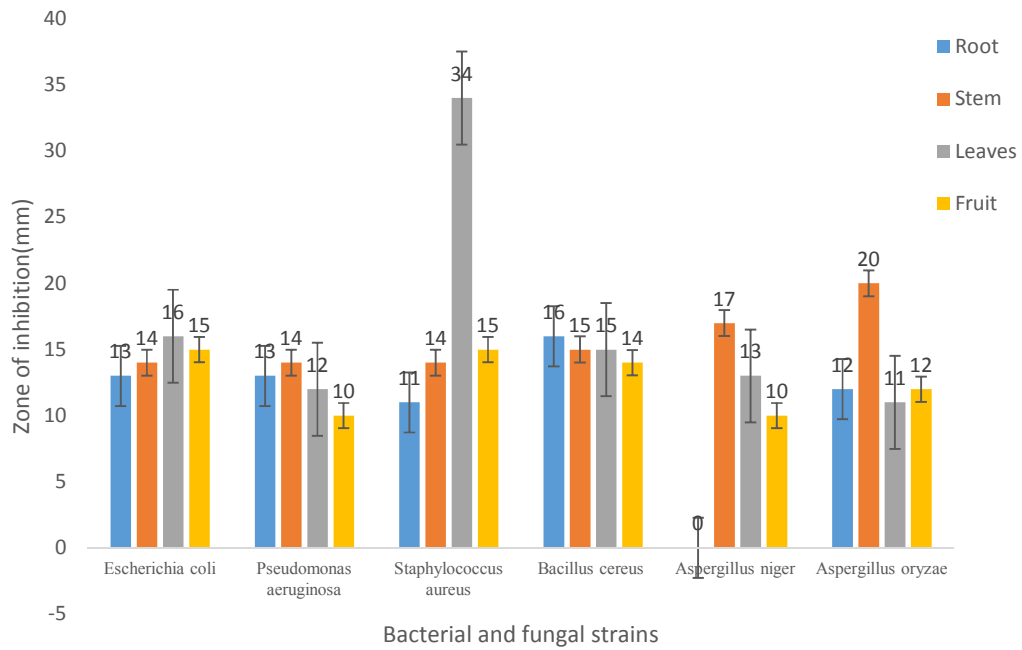


Fig. 1. Zone of inhibition of chloroform extracts

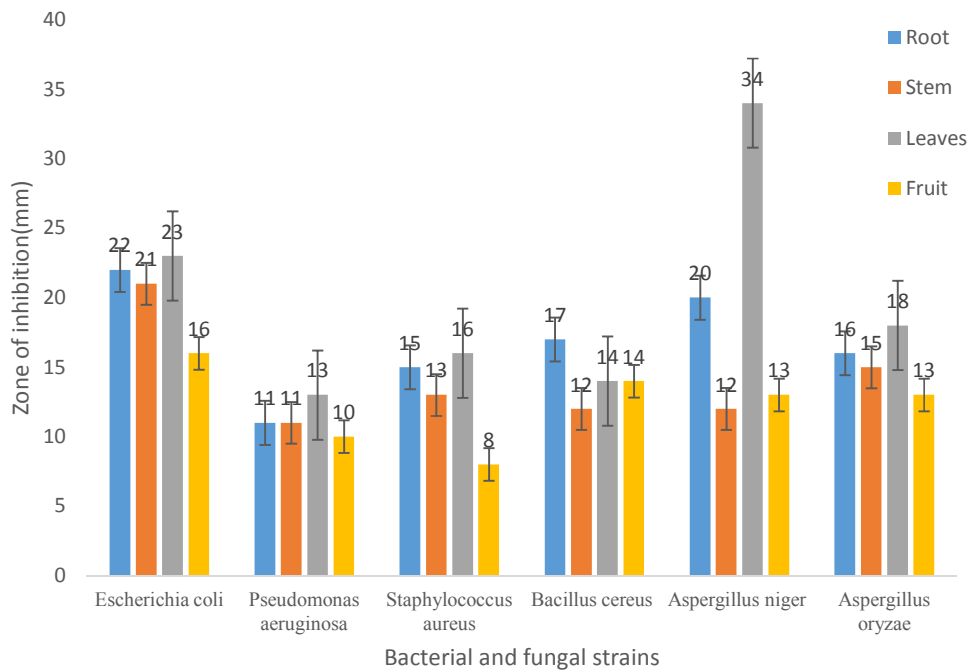


Fig. 2. Zone of inhibition of methanol extracts

Table 3. Zone of inhibition exhibited by different plant parts against bacterial and fungal strains

Plant parts	Solvents	Zone of inhibition (mm)					
		Bacterial strains				Fungal strains	
		<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Aspergillus niger</i>	<i>Aspergillus orezae</i>
Root	Chloroform	13	13	11	16	0	12
	Methanol	22	11	15	17	20	16
Stem	Chloroform	14	14	14	15	17	20
	Methanol	21	11	13	12	12	15
Leaves	Chloroform	16	12	34	15	10	11
	Methanol	23	13	16	14	34	18
Fruit	Chloroform	15	10	15	14	10	12
	Methanol	16	10	8	14	13	13

4. CONCLUSION

Different parts of plant *ficus carica* were used to investigate the antimicrobial and antifungal activity against two gram positive bacteria, two gram negative bacteria and two fungal species and concluded that high potential was observed in menthanol extract of leaves against gram negative bacteria *Escherichia coli* with zone of inhibition 23mm and fungus *Aspergillus niger* with zone of inhibition 34mm. Therefore, plant *ficus carica* can be used for the treatment of bacterial and fungal infections especially leaves have high antimicrobial and antifungal activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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