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# ANTIMICROBIAL POTENTIAL OF LEAVES OF FOUR FICUS SPECIES FROM DISTRICT BHIMBER AZAD KASHMIR, PAKISTAN

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**Abstract**

*Ficus* genus has medicinally important plant and being used by indigenous communities of Azad Kashmir. In this research, some taxa of Ficus (*F. bengalensis*, *F. carica*, *F. sermentosa,* and *F. semicordata*) were analyzed to investigate their antimicrobial potential. In antibacterial analysis three strains viz: *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* were tested using negative and positive controls. For antimycotic studies three fungi taxa: *Fusarium solani, Aspergillus flavus* and *Candida albicans* were used in compatible with both type of control set up for comparison. In analysis, plant samples were extracted by maceration method using four solvents Petroleum Ether (P.E.), Chloroform, Methanol and water. Two methods viz: Agar Well Diffusion Method and Micro dilution method. Methanol was proved to be the best solvent with highest %age yield. Highest zone of inhibition (ZI) was found for methanolic extract of lead of *Ficus bengalensis* against *S. aereus* with 22.3±0.65 mm and AI: 0.94. For fungi maximum ZI was 25.3±0.60 mm against *C. albicans*. In *Ficus bengalensis* extracts; most resistant bacterial strains was *P. aeruginosa* with MBC of 93.5±0.30 µg and most resistant fungus was *A. flavus* with MFC of 108.6±1.12µg. Among *F. carica* extracts best ZI was 20.5±0.55 mm and AI of 0.93 agaisnt *P. aeruginosa* whilst for antimycotic analysis MFC was 51.4±0.60 µg found for *F. solani*. In analysis of macerates of *F. sermentosa*; highest ZI (19.7±0.55 mm) was found for *E. coli* and ZI of 23.1±0.66 mm for *C. albicans* for methanolic extract. In its extracts, best MBC was 86.9±0.30 µg agaisnt *S. aereus* and MFC with 95.5±0.85 µg for *F. solani*. For extract analysis of *F. semicordata*, good ZI was explored for *S. aereus* with 19.5±0.80 µg and for fungus activity it was found that methanolic extract had highest ZI (21.3±0.45 mm) for *A. flavus.* In this plant activity tests, it was found that best MIC (40.5±0.60 µg), MBC (39.5±0.25µg) for *E. coli* and MFC (99.6±0.55 µg) *F. solani*. Out all analyzed taxa; methanolic extract of *F. bengalensis* was found to be best for antimicrobial dose of medication with least MBC and MFC followed by macerates of and *F. semicordata.* This research reveals that *Ficus bengalensis* and *F. semicordata* are more effective agaisnt bacteria and fungi than leaf extracts of *Ficus carica* and *F. sermentosa.* The current findings might be useful for preparation of herbal recipes or allopathic medicines by pharmaceutical industries to cure resistant gaining microbes. This will culminate into alternative medicine development by indigenous pharmacopeia for cure of these pathogens with drugs having no-side effects, synergistic in action and easy to purchase.

**Keywords:** Antibacterial activity, Antimycotic activity, MBC, MFC, Ficus taxa, Activity index

## Introduction

Plants have been used by man to cater his daily life necessities (Shinwari *et al*., 2003). The plants have been used for food and medicine by the human since his emergence on the planet (Shinwari *et al*., 2006). In recent era, plants have also been used for extraction and preparation of novel medicines by different pharmaceutical industries leading towards new drug discovery and development (Gilani *et al*., 2007). Plants potential of medicure has been acknowledged by all-time men and he has benefited from these by using their recipes in different forms to cure prevailing diseases. The indigenous use of plant based medicines by different ethnic groups have been reknown and latterly professional named as indigenous medicine system of the world like: Homeopathy, Unani, Siddha, Ayurveda and Traditional Chinese Medicines (TCMs). These systems of herbal medication have been well reputed and acknowledged by the developed world and now their revolution that herbal medicines or synergistic medicines are better than western medicines as former has less toxicity, easily availably and cost-effective for all communities of the world (Shinwari *et al*., 2005). The WHO survey reports proves that ca. 80% population of world is depending on folk and cultural medicines obtained from wild or cultivated plants by these communities. The natural medicines are time required now, because there is dare to need screen these plants for production of natural and better medicines to cure microbial diseases (Shinwari & Gilani, 2003). Currently there is dilemma arising that many microbes: i.e. bacteria and fungi are developing resistance against available allopathic medicines due to misuse of these antibiotics (Aibinu *et al.,* 2004; Aibinu *et al.,* 2003 and Anon., 2001). Due to high cost and lesser efficacy these allopathic medicines, there is trend seen in rise of mortality and morbidity rate in near future (Williams, 2000). Due to poor diagnosis by doctors and careless and irregular use of these antibiotics make them ineffective and pathogens get more resistance against these. Latterly, these medicines have adverse side effects on body and makes germs of the common diseases resistant and virulent. So, there is need for discovery of safe, effective and less or no toxic medicines and these can be easily obtained from herbal plants of the area. It proves that screening of various plants should be continued in consistency to eradicate and cure common and fatal diseases (Gilani *et al*., 2007). So, ethnopharmacological analysis and antimicrobial studies of different plant extracts is best way to explore purpose of desired drugs.

Many world scientists are working on the exploration of antimicrobial potential of different plants in view of novel drug discoveries (Moreillion *et al*., 2005; Pretorius *et al.,* 2003).

Many phytochemicals are used are extracted from different medicinal plants by using different solvents having various extraction procedures. It is reported that more than 110 organic compounds have been used for allopathic drug development around the globe. Up to now, many plants are which as whole or partially used as source of medicines to eradicate different harmful pathogens (Hussain *et al*., 2009). Among medicinal plants, *Ficus* is one of the reknowned plants being used as herbal medicine in many areas of the world. It is present in diverse habit: vines, shrubs and trees. *Ficus* is diverse genus which belongs to family Moraceae and it has more than 800 species in the world (Hameed, 2006). Ficus is generally known is as Fig tree and out these total species 500 are inhabited in Asia and 29 species indigenous to Pakistan. Frequently found taxa of the genus found in Pakistan includes *Ficus benghalensis, F. carica, F. palmata, F. elestica, and F. auriculata* and *F. religiosa* etc. Chemical screening of Ficus proved that the genus has many bioconstituents which are medicinally more important (Veberic *et al*., 2008; Abdel- Hameed, 2009; Lee *et al*., 2002; Basudan *et al*., 2005). Taxa of the genus have many pharmacological activities such as antimycotic, anticancerous (Kitajima *et al.,* 1999), anti-inflammatory (Lansky *et al.,* 2008), antijaundice, epilepsy (Betti, 2004; Noumi and Fozi, 2003). Many parts of these plants have been used in herbal therapeutics against bacillary dysentery, whooping cough, bronchitis, tonsillitis, influenza and flu by local people of the area (Abdel, 2009).

There is need of hour to conduct detailed study on these plants of the genus to explore medicinal profile or potential activity against bacteria and fungi of common occurrence and other human being clinical pathogens (Shinwari *et al*., 2009; Gilani *et al*., 2010).

The antimicrobial potential of any plant can be determined by using different extraction protocols and finding out its potential of minimum inhibitory concentration (MIC). The minimum bacterial concentration (MBC) and minimum fungal concentration (MFC) are also determined that is due to presence of different bioactive compounds in the extracts. These protocols of agar well diffusion and streak plating do possess for promising results for determining antimicrobial potential of these plants of ficus genus. The sampled plants were collected district Bhimber of Azad Jammu and Kashmir which is also called "bab-e-Kashmir" since it has been major route of entrance by prior emperors of history. This area is dynamic with plains site at its basement and covered by high and lofty mountains of peer panjal and shiwilk ranges with diverse vegetation and this constitute subtropical part of forests (Ishtiaq *et al.,* 2013). Administratively district Bhimber is divided into three subdivisions and samples of experiments from collected from all three major localities of it.

Albeit many work has been conducted on ethnobotanical data compilation but hitherto nothing exists on genus *Ficus* particularly with reference of their pharmacological investigations. Hence, it is need to test the efficacy of these plants' parts against different germs and pathogens which cause diseases in human body (Westh *et al*., 2004). There is no research work conducted on ethnopharmacology and antimicrobial potential of ficus taxa from Azad Kashmir, so this novel attempt to explore the said potential through different dedicated protocols.

The objectives of the research were multifarious constituting: (1) to determine antibacterial activity of different plant species, (2) to explore antimycotic potential of ficus taxa; (3) to find extraction potential of different solvents and determine the best solvent and extract against tested microbes and (4) to calculate MBC, MFC and Activity Index (AI) for different extracts against tested pathogens.

## Material and Methods

**Plant Sampling:** Experimental samples of selected plants of genus Ficus (*F. bengalensis*, *F. carica*, *F. sermentosa,* and *F. semicordata*) were observed to be diseased free and then collected from various sites of district Bhimber Azad Kashmir. Plants were identified by taxonomist: "Dr M Ishtiaq Ch" of the Department of Botany. Healthy and diseases free leaf samples were collected and kept in proper polythene bags as per need of experiment while three plant herbaria were prepared using standard protocols and deposited in herbarium of department for future reference.

**Media Preparation and Culturing of Microorganisms:** Tested and identified microorganisms (bacteria and fungi) in form of stock cultures were received from Department of Biotechnology, Mirpur Uni. of Sci. and Tech. Mirpur Azad Kashmir. The parent stocks of bacteria were grown on nutrient agar (NA) medium while fungi were cultured on potato dextrose agar (PDA). Then sub-culturing was prepared for both test organisms and kept at 4 $^{0}$C until next use. Series of dilutions of inoculums were grown on solidified media for confirmation of that no contamination is in it.

**Plant Extraction Procedure:** The leaves of four selected taxa of ficus genus were washed, dried and shadow dried at room temperature for one week. The dried parts were cut and powdered by using electric grinder and stored in polythene bags with tags until next use. In the analysis, four solvents were used to obtain maximum profile of phytochemicals. Petroleum ether (PE), Chloroform (Chl), Methanol (MeOH) and Water (Aq) solvents applied in gradient of polarity.

For phytochemical analysis, maceration protocol was employed and 50g of each plant was mixed/ dipped in P.E, solvent (250ml) and kept it for one week in room, with daily agitating process (Handa *et al.,* 2008). The macerate was filtered with Whatman paper after seven days and filtrate was separated in bottle while residue was dipped in Chl solvent for one week. The filtrate was dried by vacuum pump and rotary evaporator. The macerate of second solvent and other two solvents (MeOH and Aq)

were also processed in repetition as mentioned above. Each dried filtrate was weighed to find to extraction yield by each solvent for all taxa of ficus. Extracts were stored at room temperature until next use.

**Testing for antimicrobial activity**: For analysis of antibacterial potential of different extracts of selected ficus species protocols of Murray *et al.,* (1995) and Olurinola (1996) were applied with some modifications. In this experiments agar well diffusion method and measuring of minimum inhibitory concentration (MIC) was tested. During the experiment negative control (blank disc) and positive control (antibiotic disc) plates/ tests were also used for comparison of MIC, activity index (AI), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). Activity Index was calculated as: Zone of Inhibition of test sample/Zone of inhibition of standard (antibiotic used).

For antibacterial analysis, first NA medium in concentration of 40 gm/L was prepared and for antimycotic studies PDA medium with 39 gm/L concentration was prepared. All experimental materials, glassware used and prepared media (in petri dishes) was sterilized using digital autoclave at 121°C for 20 min. Then petri dishes containing NA growth media were swab with eight-hour-old broth culture of bacteria while for fungi similar process was repeated on PDA medium poured in petri dishes (PDs). In each PD wells (holes) were engraved using sterilized cork borer with 10 mm diameter and keeping adjacent distance of two centimeter. A stock solution of each plant extract of all solvents prepared with conc. of 1 mg/mL in MeOH. Then different concentrations (5µl, 10µl) of each extracts were added in well using micropipette and let it diffuse at RT for two hours. Then PDs were incubated 37°C for one day and for fungi analysis at 28°C for 28 hours. For each run experiment was repeated thrice and data were tabulated and analyzed by dedicated software.

**Determination of MIC by microdilution protocol**: MIC is the least concentration of each trial used which is able to inhibit visible growth of any microorganism, i.e. bacterium and fungus. Different serial dilutions of various concentrations were prepared for bacterial and mycotic growth and procedure was adopted as standard assay of WHO for microorganisms (2006). The bacteria and fungi are inoculated in different dilution PDs, incubated and stored for 72 hours at 28$^{O}$C, further process as per general guidelines. Determination of MIC is significant because it clarifies that how an organism/ microbe is resistant against different doses of tests applied. MIC determines that how much drug dose can be optimized for curing any bacterium or fungus with an antibiotic and this is borrowed from method of Mitscher *et al.,* (1972). For bacteria MBC and for fungi MFC were calculated by visualizing the PDs under binocular microscopes or/and colony counter following recipe of Gautam *et al*., (2007). In MBCs was determined as the concentration of extracts which can eradicate of all or nearly 99.5% of bacteria population in PDs as per comparison with positive control (tetracycline). Protocol of Mitscher *et al.,* (1972) was used for calculating the MFC, in it 2µl of each sub-culture included and incubated for 72 hours at 28$^{O}$C and then PDs were observed for finding obsolete or 99.5% killing of fungi. The fungicidal results of plants extracts were compared with positive control PD having addition of penicillin and each of experimental run was repeated thrice.

## Results

*Ficus* is diverse genus and its many plants do possess medicinal use by many populations of the world. Medicinal potential of leaves of four ficus species viz: *F. bengalensis*, *F. carica*, *F. sermentosa,* and *F. semicordata* against different bacterial and fungal pathogens was checked and analyzed. In the analysis, samples were collected from four sites of the same plant and processed for extraction. Leaf was selected for test of antimicrobial activity as it is the most active part of plant which has maximum activity of photosynthesis and it is excessive quantity of phytochemicals. For extraction, four solvents (P.E. Chl., MeOH, Aq) were used ranging from polar to non-polar and maceration procedure was found appropriate. The methanol proved to be the best solvent followed by the aqueous, chloroform and P.E. (Fig. 1). In analysis agar well diffusion, (AWD) method and micro-dilution method (MDM) were used for testing susceptibility against bacterial and fungal clinical human pathogens. For all extracts zone of inhibition (ZI) and activity index (AI) are determined and results are tabulated. The extracts were tested in comparison with standard antibiotics (penicillin and tetracycline) as positive control using concentration of 1 mg/disc.

In the antibacterial analysis of extracts of *Ficus bengalensis*, it was found that maximum ZI (22.3±0.65) was found against *S. aereus* with 0.94 AI. For antimycotic test highest values of ZI was 25.3±0.60 mm against *C. albicans* having AI of 1.55 (Table 1; Figs. 2 & 3). For *F. bengalensis*, the best minimum bactericidal concentration (MBC) was determined to be 83.7±0.90 mg against *S. aereus* strain for in macerate of methanolic extract. Whilst minimum fungicidal concentration (MFC) was found for the extract of methanolic fraction of *F. bengalensis* leaf against *F. solani* with 91.4±0.40 mg (Table 2; Fig. 5).

For *Ficus carica* leaf's antimicrobial analysis depicted that in exploration of antibacterial potential of different macerates; maximum ZI was found against *P. aeruginosa* with 20.5±0.55 mm and AI of 0.93, followed by P.E. extract of leaf against *S. aereus* with ZI of 17.1±0.75 mm and AI of 0.72 (Table 3, Fig. 2 & 3). As water extract produced second best yield of phytochemicals similarly highest findings were calculated for MIC (49.5±0.77), MBC (99.3±0.50) and MFC (112.7±0.74). *P. aeruginosa* was found to be most resistant bacterial strain which had highest MBC 105.6±0.09 mg in aqueous macerate (Table 4; Fig. 4). F. solani proved to be most resistant species of fungus which can be killed at MFC with 122.2±0.40 using PE extract and most susceptible fungal species was C. albicans with showed positive MFC at 52.5±0.80 mg (Table 4; Fig. 5).

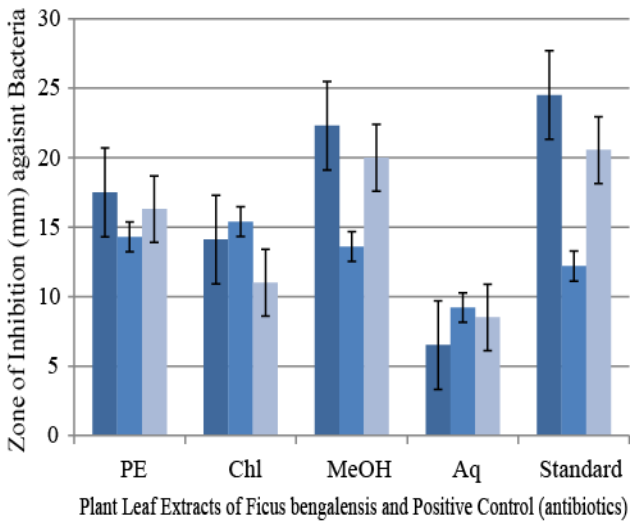


Fig. 1: % age Yield Extraction of Leaf of *Ficus bengalensis* using four solvents

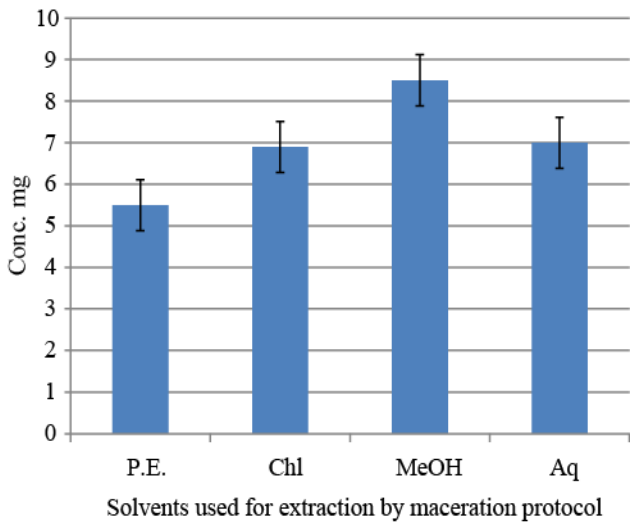


Fig. 2. Zone inhibition (mm) of Leaf extract of *Ficus bengalensis* using four solvents against three bacterial strains

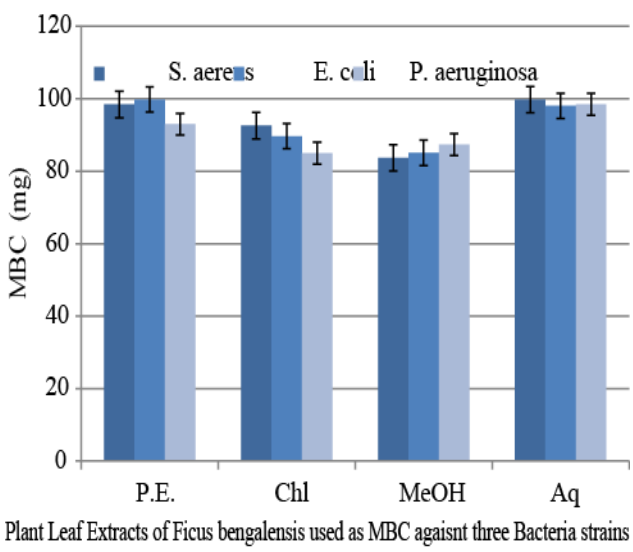


Fig. 3. Zone inhibition (mm) of Leaf extract of *Ficus bengalensis* using four solvents against three Fungal species

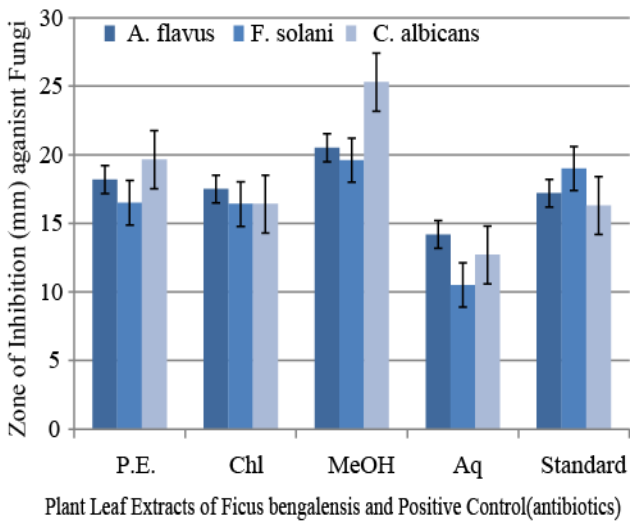


Fig. 4: MBC of three Bacterial strains for Leaf extract of *Ficus bengalensis* using four solvents

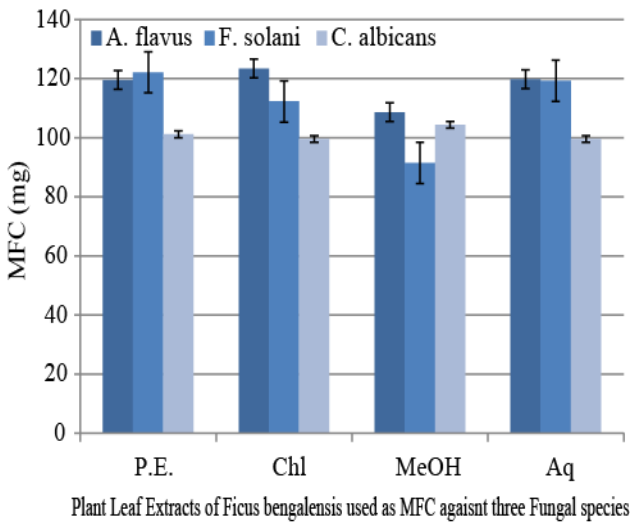


Fig. 5: MFC of three Fungal species for Leaf extract of *Ficus bengalensis* using four solvents

For extracts of *F. sermentosa*; it was found that methanol was the best solvent with good yield of extraction (Fig. 1). In comparison of all macerates of leaf of *Ficus sermentosa* it was found that *E. coli* was the most resistant bacterial strain with ZI of 19.7±0.55 mm and AI of 1.38; followed by *S. aereus* with ZI of 19.1±0.90 mm and AI of 0.94 (Table 5). In antimycotic analysis, it was found that *C. albicans* was the most resistant species among all tested pathogenic fungi with ZI of 23.1±0.66 mm agsint methanolic extract and having AI of 1.18. The least resistant taxon of fungi tested was *F. solani* with ZI of 21.5±0.80 mm and AI of 1.04 (Table 5). For second test extracts of *F. sermentosa*; it was explored that best MBC was 83.7±0.80 µg was against E. coli and it was found to be the least resistant bacteria. *P. aeruginosa* was the most resistant strain of bacteria for all macerates of leaf and out these its highest values were found for chloroform extract with MBC 92.5±0.55 µg (Table 6). In fungal tests; it was depicted by the all strains that P.E. extract was best one with MFC 114.4±0.40 µg against *F. solani,* followed by *A. flavus* with 113.4±0.45 µg for MFC. It was found that C.

albicans was least resistant species and methanolic extract showed best MFC 99.8±0.90 µg (Table 6).

The species *Ficus semicordata* depicted the good and promising results against different bacterial and fungal species. In antibacterial exploration, it was found that methanolic extract produced best ZI against *S. aereus* with 19.5±0.80 mm and AI of 0.92. In this test, *P. aeruginosa* was found to be the most susceptible strain of bacterial against different macerates of leaf of *Ficus semicordata*, and it depicted ZI of 16.4±0.90 mm and AI of 0.72 (Table 7). In antimycotic research analysis for the species, it was determined that *A. flavus* was the most susceptible taxon having highest ZI of 21.3±0.45 mm and AI of 1.18. It was followed by the *C. albicans* with 20.4±0.80 mm against MeOH extract with production of activity index (AI) of 1.02. The least values of ZI were 18.5±0.10 mm for *F. solani* (Table 7). It was found that methanolic extract was best in potential followed by water extract and then chloroform macerate (Table 7; Fig. 1).

Highest MIC and MBC were found for *S. aereus* with 40.5±0.60 µg and 84.8±0.85 µg, respectively. In comparison of all solvent's macerates it was determined that *P. aeruginosa* was found to be most resistant bacterial strain with MBC: 101.5±0.80 in water; 99.2±0.75 in PE; 98.7±0.50 in chloroform and 95.9±0.30 in MeOH respectively (Table 8). For antimycotic analysis, it was depicted that against *A. flavus* species methanolic extract was the best one with MFC having 114.4±0.30 µg, followed by aqueous macerate with MFC 115.9±0.60 µg, for chloroform extract its MFC was 118.6±0.45 µg and for PE extract its MFC was 119.3±0.90 µg Table 8). These results proved that methanolic extract was the best one to kill or control its growth with MFC values of 114.4±0.30 µg and same findings proved by %age yield graph (Fig. 1).

**Table 1. Zone of Inhibition (ZI) and Activity Index (AI) of leaf extracts of *Ficus bengalensis* against three bacterial strains and three fungal species in four different solvents.**

| Test Organism / Solvents | Bacteria | | | | | | Fungi | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aereus* | | *E. coli* | | *P. aeruginosa* | | *A. flavus* | | *F. solani* | | *C. albicans* | |
| | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI |
| P.E. | 17.5±0.55 | 0.71 | 14.3±0.50 | 1.17 | 16.3±0.10 | 0.79 | 18.2±0.50 | 1.05 | 16.5±0.20 | 0.86 | 19.65±0.30 | 1.20 |
| Chl | 14.1±0.40 | 0.57 | 15.4±0.10 | 1.26 | 11±0.90 | 0.53 | 17.5±0.20 | 1.01 | 16.4±0.85 | 0.86 | 16.4±0.25 | 1.00 |
| MeOH | 22.3±0.65 | 0.94 | 13.6±0.45 | 1.11 | 20±0.55 | 0.97 | 20.5±0.10 | 1.19 | 19.6±0.84 | 1.03 | 25.3±0.60 | 1.55 |
| Aq | 6.5± 0.10 | 0.26 | 9.2± 0.35 | 0.75 | 8.5± 0.75 | 0.41 | 14.2±0.50 | 0.82 | 10.5±0.65 | 0.55 | 12.7±0.55 | 0.77 |
| Positive Control (Antibiotics Used) | 24.5 | | 12.20 | | 20.55 | | 17.20 | | 19.00 | | 16.30 | |

Key: ZI =zone of Inhibition; AI=activity index; PE=Petroleum ether; Chl=Chloroform; MeOH=Methanol; Aq=Water/Aqueous; All readings are taken in triplicate expressed in S.E.M.

**Table 2. MIC, MBC and MFC of leaf extracts of *Ficus bengalensis* against three bacterial strains and three fungal species in four different solvents.**

| Test organism / Solvent | Bacteria | | | | | | Fungi | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aereus* | | *E. coli* | | *P. aeruginosa* | | *A. flavus* | | *F. solani* | | *C. albicans* | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MFC | MIC | MFC | MIC | MFC |
| P.E. | 51.5±0.20 | 98.5±0.70 | 41.2±0.20 | 99.8±0.75 | 47.5±0.65 | 93.5±0.30 | 55.5±0.90 | 119.5±0.11 | 62.1±0.25 | 122.1±0.15 | 59.5±0.60 | 101.1±0.80 |
| Chl | 53.2±0.80 | 92.6±0.20 | 48.4±0.50 | 89.7±0.88 | 41.5±0.20 | 85.4±0.10 | 53.6±1.04 | 123.4±0.56 | 59.1±0.70 | 112.2±0.77 | 57.9±0.20 | 99.5±0.40 |
| MeOH | 40.5±0.10 | 83.7±0.90 | 42.6±0.30 | 85.1±0.57 | 48.2±0.66 | 87.4±0.20 | 52.8±0.85 | 108.6±1.12 | 51.2±0.90 | 91.4±0.40 | 45.8±0.20 | 104.3±0.30 |
| Aq | 52.6±1.55 | 99.8±0.95 | 48.9±0.55 | 98.1±0.65 | 47.5±0.32 | 98.5±0.30 | 58.8±0.95 | 119.7±0.81 | 61.5±0.20 | 119.2±0.60 | 46.7±0.10 | 99.5±0.30 |

Key: MIC=minimum inhibitory concentration; MBC= minimum bactericidal concentration; MFC= minimum fungicidal concentration; P.E.= petroleum ether; Chl=Chloroform; MeOH=Methanol; Aq=Water/Aqueous; All readings are taken in triplicate expressed in S.E.M.

**Table 3. Zone of inhibition (ZI) and Activity Index (AI) of leaf extracts of *Ficus carica* against three bacterial strains and three fungal species in four different solvents.**

| Test organism / Solvent | Bacteria | | | | | | Fungi | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aereus* | | *E. coli* | | *P. aeruginosa* | | *A. flavus* | | *F. solani* | | *C. albicans* | |
| | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI |
| P.E. | 17.1±0.75 | 0.72 | 16.1±0.55 | 1.11 | 14.2±0.60 | 0.64 | 18.3±0.90 | 0.98 | 13.4±0.30 | 0.61 | 18.4±0.50 | 1.05 |
| Chl | 15.2±0.40 | 0.64 | 14.2±1.55 | 0.97 | 17.3±0.90 | 0.78 | 16.7±0.80 | 0.90 | 18.6±0.40 | 0.85 | 19.6±0.70 | 1.12 |
| MeOH | 16.2±0.80 | 0.68 | 15.4±0.20 | 1.06 | 20.5±0.55 | 0.93 | 19.3±0.10 | 1.04 | 19.5±0.30 | 0.89 | 21.8±0.95 | 1.24 |
| Aq | 10.5±1.00 | 0.44 | 11.3±0.10 | 0.77 | 13.1±0.20 | 0.59 | 14.2±0.20 | 0.76 | 13.1±0.60 | 0.60 | 14.3±0.40 | 0.81 |
| Positive Control (Antibiotics Used) | 23.55 | | 14.50 | | 21.90 | | 18.55 | | 21.75 | | 17.50 | |

Key: ZI =zone of Inhibition; AI=activity index; PE=Petroleum ether; Chl=Chloroform; MeOH=Methanol; Aq=Water/Aqueous; All readings are taken in triplicate expressed in S.E.M.

**Table 4. MIC, MBC and MFC of leaf extracts of *Ficus carica* against three bacterial strains and three fungal species in four different solvents.**

| Test organism / Solvent | Bacteria | | | | | | Fungi | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aereus* | | *E. coli* | | *P. aeruginosa* | | *A. flavus* | | *F. solani* | | *C. albicans* | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MFC | MIC | MFC | MIC | MFC |
| P.E. | 52.5±0.60 | 86.4±0.40 | 49.3±0.55 | 92.2±0.60 | 48.4±0.70 | 101.3±0.45 | 62.5±0.80 | 105.3±0.40 | 61.3±0.40 | 122.2±0.40 | 60.9±0.40 | 101.7±.0.50 |
| Chl | 49.5±0.77 | 89.5±0.85 | 47.2±0.30 | 93.2±0.10 | 48.9±0.10 | 103.2±0.40 | 57.5±0.65 | 109.6±0.70 | 60.9±0.45 | 118.3±0.10 | 59.4±0.50 | 103.9±0.40 |
| MeOH | 46.1±0.55 | 88.8±0.15 | 41.9±0.25 | 82.4±0.30 | 41.3±0.90 | 98.5±0.80 | 57.2±0.70 | 102.3±0.20 | 51.4±0.60 | 102.4±0.55 | 52.5±0.80 | 99.6±0.45 |
| Aq | 56.6±0.44 | 99.3±0.50 | 49.1±0.90 | 98.2±0.55 | 52.4±0.70 | 105.6±0.09 | 58.5±0.20 | 112.7±0.74 | 61.3±0.40 | 120.5±0.95 | 59.1±0.25 | 102.5±0.55 |

Key: MIC=minimum inhibitory concentration; MBC= minimum bactericidal concentration; MFC= minimum fungicidal concentration; P.E.= petroleum ether; Chl=Chloroform; MeOH=Methanol; Aq=Water/Aqueous; All readings are taken in triplicate expressed in S.E.M.

**Table 5. Zone of inhibition (ZI) and Activity Index (AI) of leaf extracts of *Ficus sermentosa* against three bacterial strains and three fungal species in four different solvents.**

| Test organism / Solvent | Bacteria | | | | | | Fungi | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aereus* | | *E. coli* | | *P. aeruginosa* | | *A. flavus* | | *F. solani* | | *C. albicans* | |
| | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI |
| P.E. | 16.5±0.80 | 0.73 | 15.3±0.70 | 1.07 | 14.6±0.66 | 0.69 | 20.1±0.55 | 1.10 | 18.5±0.50 | 0.90 | 18.7±0.90 | 0.95 |
| Chl | 15.3±0.30 | 0.67 | 14.7±0.20 | 1.03 | 17.3±0.10 | 0.82 | 19.7±0.90 | 1.07 | 19.7±0.26 | 0.96 | 17.5±0.80 | 0.89 |
| MeOH | 19.1±0.90 | 0.84 | 19.7±0.55 | 1.38 | 18.8±0.30 | 0.89 | 22.4±0.90 | 1.22 | 21.5±0.80 | 1.04 | 23.1±0.66 | 1.18 |
| Aq | 9.2±0.60 | 0.40 | 10.9±0.20 | 0.76 | 11.5±0.90 | 0.54 | 16.3±0.70 | 0.89 | 13.5±0.45 | 0.65 | 11.5±0.50 | 0.58 |
| Positive Control (Antibiotics Used) | 22.50 | | 14.25 | | 21.00 | | 18.25 | | 20.50 | | 19.50 | |

Key: ZI =zone of Inhibition; AI=activity index; PE=Petroleum ether; Chl=Chloroform; MeOH=Methanol; Aq=Water/Aqueous; All readings are taken in triplicate expressed in S.E.M.

**Table 6. MIC, MBC and MFC of leaf extracts of *Ficus sermentosa* against three bacterial strains and three fungal species in four different solvents.**

| Test Organism / Solvent | Bacteria | | | | | | Fungi | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aereus* | | *E. coli* | | *P. aeruginosa* | | *A. flavus* | | *F. solani* | | *C. albicans* | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MFC | MIC | MFC | MIC | MFC |
| P.E. | 52.5±0.50 | 91.3±0.4 | 47.1±0.40 | 97.5±0.58 | 47.9±0.45 | 93.6±0.87 | 59.5±0.90 | 113.4±0.45 | 60.6±0.20 | 114.4±0.40 | 58.5±0.80 | 103.6±0.70 |
| Chl | 53.6±0.55 | 89.4±0.55 | 46.9±0.66 | 98.5±0.59 | 46.2±0.40 | 92.5±0.55 | 59.2±0.60 | 111.9±0.50 | 59.5±0.60 | 115.8±0.50 | 58.6±0.20 | 101.5±0.55 |
| MeOH | 50.2±0.90 | 86.9±0.30 | 42.6±0.65 | 83.7±0.80 | 45.8±0.30 | 96.5±0.65 | 57.1±0.70 | 99.3±0.48 | 51.2±0.50 | 95.5±0.85 | 54.3±0.50 | 99.8±0.90 |
| Aq | 55.8±0.95 | 101.6±0.65 | 49.5±0.95 | 99.5±0.25 | 48.1±0.70 | 101.7±0.80 | 62.6±0.90 | 112.2±0.20 | 63.4±0.30 | 114.0±0.55 | 63.5±0.90 | 106.4±0.50 |

Key: MIC=minimum inhibitory concentration; MBC= minimum bactericidal concentration; MFC= minimum fungicidal concentration; P.E.= petroleum ether; Chl=Chloroform; MeOH=Methanol; Aq=Water/Aqueous; All readings are taken in triplicate expressed in S.E.M.

**Table 7. Zone of Inhibition (ZI) and Activity Index (AI) of leaf extracts of *Ficus semicordata* against three bacterial strains and three fungal species in four different solvents.**

| Test Organism / Solvent | Bacteria | | | | | | Fungi | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aereus* | | *E. coli* | | *P. aeruginosa* | | *A. flavus* | | *F. solani* | | *C. albicans* | |
| | ZI (mm) | AI | ZI(mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI | ZI (mm) | AI |
| P.E. | 16.5±0.55 | 0.78 | 14.4±0.7 | 0.96 | 16.6±0.70 | 0.73 | 19.6±0.20 | 1.08 | 16.7±0.44 | 0.87 | 19.5±0.90 | 0.97 |
| Chl | 18.3±0.45 | 0.87 | 15.6±0.45 | 1.04 | 15.3±0.44 | 0.68 | 18.6±0.55 | 1.03 | 15.8±0.55 | 0.83 | 18.5±0.50 | 0.92 |
| MeOH | 19.5±0.80 | 0.92 | 18.6±0.95 | 1.24 | 16.4±0.90 | 0.72 | 21.3±0.45 | 1.18 | 18.5±0.10 | 0.97 | 20.4±0.80 | 1.02 |
| Aq | 18.5±0.55 | 0.87 | 10.5±0.35 | 0.70 | 10.2±0.20 | 0.45 | 14.6±0.50 | 0.81 | 10.5±0.47 | 0.55 | 12.9±0.70 | 0.64 |
| Positive Control (Antibiotics Used) | 21.00 | | 15.00 | | 22.50 | | 18.00 | | 19.00 | | 20.00 | |

Key: ZI =zone of Inhibition; AI=activity index; PE=Petroleum ether; Chl=Chloroform; MeOH=Methanol; Aq=Water/Aqueous; All readings are taken in triplicate expressed in S.E.M.

**Table 8. MIC, MBC and MFC of leaf extracts of *Ficus semicordata* against three bacterial strains and three fungal species in four different solvents.**

| Test Organism / Solvent | Bacteria | | | | | | Fungi | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aereus* | | *E. coli* | | *P. aeruginosa* | | *A. flavus* | | *F. solani* | | C. albicans | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MFC | MIC | MFC | MIC | MFC |
| P.E. | 47.5±0.50. | 85.5±0.55. | 47.1±0.70 | 96.1±0.66 | 41.5±0.20 | 99.2±0.75 | 59.4±0.90 | 119.3±0.90 | 62.5±0.77 | 119.5±0.95 | 59.5±0.78 | 99.5±0.60 |
| Chl | 46.2±0.40 | 83.5±0.50 | 46.8±0.50 | 90.5±0.20 | 40.8±0.60 | 98.7±0.50 | 56.8±0.27 | 118.6±0.45 | 61.5±0.90 | 117.5±0.50 | 58.3±0.50 | 104.2±0.55 |
| MeOH | 40.5±0.60 | 84.8±0.85 | 39.5±0.25 | 81.8±0.40 | 43.5±0..30 | 95.9±0.30 | 55.3±0.70 | 114.4±0.30 | 51.8±0.68 | 99.6±0.55 | 56.3±0.85 | 105.8±0.90 |
| Aq | 47.2±0.40 | 94.3±0.30 | 43.4±0.15 | 92.5±0.60 | 47.3±0.15 | 101.5±0.80 | 61.5±0.25 | 115.9±0.60 | 64.5±0.18 | 121.5±0.60 | 61.4±0.55 | 109.3±0.45 |

Key: MIC=minimum inhibitory concentration; MBC= minimum bactericidal concentration; MFC= minimum fungicidal concentration; P.E.= petroleum ether; Chl =Chloroform; MeOH=Methanol; Aq=Water/Aqueous; All readings are taken in triplicate expressed in S.E.M.

## Discussion

The plants have been used as source of herbal medicines since long past times (Qasim *et al*., 2010). The people of different areas and cultures of the world have been depending on plants for curing different infectious diseases. Most commonly diseases are caused by pathogens like viruses, bacteria and fungi (Shinwari & Qaiser, 2011). As man cannot depend on allopathic medicines solely; as people of rural areas where of the populations of developing countries dwell, cannot buy it or cannot approach it easily. So, they depend on botanic drugs for such treatments of ailments; and plants do possess various phytochemicals and that are rich source of diseases eradication (Kelmanson *et al*., 2000). So, there is need of hour to explore alternatives of westernized medicines, which might be cheaper, without or with least side effects, cost effective and having easy to use and approachable (Ahmad & Beg, 2001). This is also inevitable to use plants as source of medicines as many pathogens are becoming resistant against available antibiotics (Guleria *et al*., 2006). In past many plants have been test for as alternative source of drug discovery and drug development (Zakaria *et al*., 2007).

*Ficus* taxa are very promising plants for curing many bacterial or fungal based diseases as these plant recipes are in-culture since time immemorial. The ficus plant are also mentioned holy book of Quran as plant of heaven and that can give good cure and potency for different diseases (Rahman *et al*., 2011). In the results of use of four different solvents, it was found that use of polar and non-polar solvent reveals good range of chemical compounds. The maceration protocol was the best and easy to use and it gave promising results as it was mentioned in past work (Ilango *et al*., 2009). On the average it was found that methanol was the best solvent with best %age yield shown (Fig. 1) and it is incoincdence with past research findings (Geethalakshmi *et al*., 2010). It was declared that methanolic macerate produced good antimicrobial result against all test pathogens and same reports were published by Upadhyay *et al*., (2011).

The methanolic extract was the best one and proved significantly effective against all resistant and non-resistant microbial strains (Preethi *et al*., 2010; Seyydnejad *et al*., 2010). It is due to the fact that organic solvents can ooze out maximum organic compounds from samples of plants and these extracts depicted good bacteriostatic and bactericidal activity. These finding are in-line with past results of Cowan (1999) that organic phytochemicals are extracted out in saturated organic solvents.

In research work of this project, it was found that in methanolic extract of F. bengalensis, maximum ZI and AI was determined against all bacterial and fungal species. It had also been wrote by past workers that methanolic extract proved to be the best and effective antimicrobial agent then all other solvents (Sekar *et al*., 2012) and it is mentioned in Fig. 1 and Tables 1,3,5,7, respectively.

It was found that *P. aeruginosa* and *C. albicans* were the most resistant bacterial and fungal species, respectively when test against various leaf macerates. The least effect of macerates was found on *S. aerus* and *F. soleni* species and that might be their easy vulnerability agaisnt various macerates. In the analysis it was found that P.E. and cholorformic extracts were shown as moderate in antimicrobial activity for all test species of Ficus and similar type of words have been reported by previous researchers (Murugesan *et al*., 2011). Still their efficacy as good solvent cannot be denied as shown in results of four test taxa of Ficus in different Tables (1,3,5, 7; Figs. 2,3) and Thatoi *et al*., (2008) also cited that these solvents are good for extraction of herbs for antimicrobial analysis.

The methodologies used in the research comprises of agar diffusion well method and micro-dilution method, both were found to be good to determine MIC, MBC and MFC for all clinical pathogens under investigation and same methodologies have been used by past workers for analyzing antibacterial and antimycotic activity of different plants (Arora *et al*., 2007). The good range of MBC and MFC for all plants of Ficus proved that at higher concentration it acted as microbiocidal and at moderate or low dose it functioned as microbiostatic agent, which retards the growth of pathogens and similar findings had been reported by past microbiologists (Pavithra *et al*., 2010; Gurudeeban *et al*., 2010). These parameters of MIC, MBC and MFC are good features for testing in lab or clinical centers to test resistance of pathogens against different medicines (antibiotics) and then alternative's search is paramount that can be best cure for these diseases. *Ficus* species have been reported good source of microbiocidal but this work is sporadic and scarcity which needs more work and thorough exploration (Alikan *et al.,* 2011). This was one the attempts to provide comparative analysis antimicrobial analysis of leaf powder of four *Ficus* taxa which have been used source of rural tonics and indigenous medicines around the world (Maji *et al*., 2010).

## Conclusion

The gist of the research work and this article is that all four taxa of Ficus have good potential to be used as antimicrobial agents to control different pathogens like bacteria and fungi. The methanol was the best solvent with all plant samples. Maximum ZI and AI were found agaisnt *P. aeruginosa* and F. solani. Ficus bengalensis proved to be the good plant as to control bacterial and mycotic infections. These findings may be used for drug discovery model in pharmaceutical industries. These out-puts provide clues for future research on all parts of Ficus species and inclusion of all or maximum taxa from Azad Kashmir. It will provide good way to discovery and drug development for future.

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