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Assessment of Antimicrobial Activity of Ethanol Solvent Extract of Medicinal Plants

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Abstract

Aim- Evaluation of the antimicrobial activity of ethanol solvent extracts of medicinal plants. **Method-** Ethanol extract of leaves of 7 plants belonging to different families were evaluated for *in vitro* antimicrobial activity against 4 bacteria and 4 fungi using agar dilution method. **Results-** *M. azedarach* were strongly inhibited the growth of *S. aureus, E. coli and P. aeruginosa* whereas *N. oleander* has strongly inhibited the growth of *B. subtilis against bacteria. M. azedarach* has maximum antibacterial and antifungal activity in comparative to other plants. *P. stratiotes and E. crassipes* have minimum activity against all other plants on antibacterial and antifungal activity respectively. The most susceptible bacterium was *E. coli* and the most resistant was *B. subtilis*. **Conclusions-** All these plants have therapeutic applications and are extremely efficient against bacterial and fungal infections. These plants can be further subjected to isolation of the therapeutic antimicrobials and to further pharmacological evaluation.

Keywords
Antifungal, antibacterial, antimicrobial, resistant and therapeutic

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INTRODUCTION

Plant extracts were used as a traditional way to treat numerous health conditions for generations after generations [1]. For several plants and plant sections, including root, stem, leafy leaves, seeds and flowers have been extensively documented antimicrobial properties over the past two decades [2]. Synthetic drugs are costly and inefficient for the curing disease but most even with side effects and adulterations [3]. Traditional medicine's popular success has led to the quest for new chemotherapy solutions for removing drug-resistant microbes and minimising antibiotic harm [4]. In India all parts of the population use medicinal plants as folk remedies or in various primaeval medical systems or in the pharmaceutical preparations of modern medicines. The National Health Experts estimate 2000 plants are used for both domestic and international use in India alone for medicinal preparations [4].

A broad spectrum of secondary metabolism such as tannins, terpenoids, alkaloids, flavonoids, phenols, and quinones are abundant in medicinal plants [5] and used worldwide for treating several diseases and infections in traditional medicine [6]. Many studies around the globe have shown that these plants and their extract have multi-antibiotic properties [7]. Rigveda, Yajurveda and Atharveda listed 67, 81 and 290 plants respectively which having therapeutic effects [8]. More than 20,000 varieties of medicinal plants have recently been documented by the World Health Organisation. Researchers constantly look for new avenues for effective medicines against cancer, tuberculosis, HIV, Skin infection and other viral and microbial infections in folk medicine [9]. Indian herbs and their extracts are being used to manage various

illnesses such as catarrh, bronchitis, pneumonia, ulcers and diarrhoea [10]. This condition provided to the search for new antimicrobials from different sources, such as medicinal plants [11]. That's why, new infection control approaches are needed to control microbial infections [12].

In the present work, 7 different medicinal plants belonging to different families were evaluated for their antibacterial properties. In addition, several plant species were not studied or identified for potential medicinal value [13]. This research has therefore been conducted to determine the biological and antimicrobial activity of the most used plant in India used in medicines, food and spices and flavouring on gram-positive standard strains bacteria represented by *Staphylococus aureus* and *Bacillus subtilis* and gram-negative standard represented by *Escherichia coli* and *Pseudomonas aeruginosa* and pathogenic fungi *Aspergillus niger, Aspergillus flavus* and *Fusarium oxisporum* [14].

MATERIALS AND METHODS

Antimicrobial Activity

Antimicrobial activity of *Eichhornia crassipes*, *Pistia stratiotes*, *Phyllanthus amarus*, *Melia azedarach*, *Nerium oleander*, *Thevetia peruviana* and *Tinospora cordifolia* was studied with their ethanolic extracts. Four bacterial and four fungal strains were selected for the primary screening.

Sources of Test Organisms

a. Bacteria

The bacterial strains *Bacillus subtilis* (MTCC 0121) (Gram+ve), *Staphylococcus aureus* (MTCC 0737) (Gram+ve), *Escherichia coli* (MTCC No. 1687) (Gram –ve) and *Pseudomonas aeruginosa* (MTCC 7925) (Gram -ve) are procured from The Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India.

b. Fungal Culture

The fungal strains *Aspergillus niger* (MTCC 1344), *Aspergillus flavus* (MTCC 9029), *Fusarium oxisporum* (MTCC6659) and *Rhizopus stolonifer* (MTCC2591) are procured from The Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India.

Culture of test microbes

The agar well diffusion assay technique was used, 500 µl of microbe's cultures age18 - 24 h were added to petri plates and nutrient agar (NA) was poured [15]. After media were solidified, holes were made by using 5 mm cork borer each hole was filled with 50 µl of plant extract. The inoculated agar plates were left in refrigerator for one hour for proper diffusion then plates were incubated, at 37°C for the bacteria for 24 h. However, for the cultivation of fungi, Potato dextrose agar (PDA) medium was prepared, and the fungal cultures were maintained on this medium by regular subcultures and the test fungi were incubated at 27°C for 48 hrs. Negative and positive controls were used. The zones of inhibition were then recorded in millimetres.

Determination of antimicrobial activity

Nutrient Agar (NA) plates were seeded with 8 h broth culture of different bacteria while potato Dextrose Agar (PDA) plates were seeded with spore suspension of fungi. A 16 h broth culture of *C. albicans* was used to seed PDA plates. In each of these plates, 2 wells (10 mm) were cut out using sterile cork borer. Using sterilized dropping pipettes, 0.3 ml of extract was carefully added into the wells and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 37°C for 18–24 h for bacterial pathogens and 3 days for fungal pathogens. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in triplicate and the mean of the diameter of the inhibition zones was calculated.

Preparation of Plates

To prepare the test plates, for both bacteria and fungi, 10 to 15 ml of the respective medium was poured into the petri dishes under aseptic conditions. They were then permitted to set at room temperature and were dried so that no drops of moisture remain on the surface. For assessing the bactericidal efficacy, a fresh suspension bacterium was prepared in saline solution from a freshly grown agar slant, while for fungicidal efficacy, a uniform spread of the test fungi was made using sterile swab.

Preparation of test extracts

Powdered of leaves of different plants were soxhlet extracted with ethanol. Similarly, 10 gm of different plant parts were homogenized separately with ethanol only and left overnight at the room temperature [16]. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled and dried in vacuum. All these fractions were stored at 4°C in a refrigerator until screened and fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

STATISTICAL ANALYSIS

Data are expressed as the mean ± SEM of at least three independent experiments. All columns (ZOI) were compared with standard using Tukey test after ANOVA using ezanova software.

RESULTS

The antimicrobial potential of both the experimental plants were evaluated according to their zone of

inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards, *viz*., Ampicillin (1.0 mg/disc), Flucanazole (1.0 mg/disc) for bacteria and fungi, respectively. The results revealed that all the extracts are potent antimicrobials against all the microorganisms studied.

(i) *Melia azedarach* L.

For all the tested microorganisms in Ethanol showed maximum antibacterial activity. In this extract maximum inhibition zone diameter was obtained in *P. aeruginosa, E. coli and Staphylococcus aureus* with diameter 22.3±0.42, 19.6±0.65 and 19.5±0.52 mm and their activity index 1.08, 1.05 and 1.02 respectively. Minimum activity showed against *Bacillus subtilis* with activity index 0.54 (Table1& 2; Figure 1; Graph. 1).

Table 1: Antibacterial bioassay of some medicinal plants in ethanol extract

Pathogenic bacteria	Zone of Inhibition (ZOI) (mm)							
	Standard	*Melia azedarach*	*Nerium oleander*	*Phyllanthus amarus*	*Thevetia peruviana*	*Tinospora cordifolia.*	*Eichhornia crassipes*	*Pistia stratiotes*
Staphylococcus aureus	19.07±0.33	19.5±0.52**	15.5±0.24**	16.3±0.91**	18.6±0.23**	12.50±0.44**	8.67±0.47**	8.00±0.00*
Bacillus subtilis	24.5±0.90	13.4±0.56**	19.6±0.73**	16.4±0.82**	9.3±0.52**	16.50±0.44**	10.00±0.81**	9.33±0.47**
Escherichia coli	18.6±0.28	19.6±0.65**	18.4±0.50**	19.2±0.32**	14.3±0.57*	10.24±0.52**	8.67±0.47**	9.00±0.81**
Pseudomonas aeruginosa	21.5±0.83	22.3±0.42**	14.3±0.34**	15.8±0.45**	17.3±0.35*	12.50±0.44**	9.67±0.47**	8.33± 0.47*

Standards: Ciprofloxacin; Activity index = ZOI of test sample/ZOI of standard. Mean±SE, * p< 0.05, ** p<0.01, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software.

Table 2: Activity index of antibacterial bioassay of some medicinal plants in ethanol extract

Pathogenic bacteria	Activity Index (AI) (mm)							
	Standard	*Melia azedarach*	*Nerium oleander*	*Phyllanthus amarus*	*Thevetia peruviana*	*Tinospora cordifolia.*	*Eichhornia crassipes*	*Pistia stratiotes*
Staphylococcus aureus	19.07±0.33	1.02	0.81	0.85	0.97	0.65	0.45	0.41
Bacillus subtilis	24.5±0.90	0.54	0.79	0.66	0.37	0.67	0.40	0.38
Escherichia coli	18.6±0.28	1.05	0.98	1.03	0.76	0.55	0.46	0.48
Pseudomonas aeruginosa	21.5±0.83	1.08	0.71	0.78	0.78	0.58	0.44	0.38

Activity index = ZOI of test sample/ ZOI of standard.

Table 3: Antifungal bioassay of some medicinal plants in ethanol extract

Pathogenic fungi	Zone of Inhibition (ZOI) (mm)							
	Standard	*Melia azedarach*	*Nerium oleander*	*Phyllanthus amarus*	*Thevetia peruviana*	*Tinospora cordifolia*	*Eichhornia crassipes*	*Pistia stratiotes*
Aspergillus niger	17.6±0.30	22.7±0.11**	20.3±0.51**	15.3±0.75**	12.9±0.66**	10.34±0.05*	8.00±0.81**	9.67±0.471**
Aspergillus flavus	17.03±0.63	21.2±0.32**	16.32±0.34**	15.8±0.32**	15.5±0.75**	16.62±1.32**	10.33±0.88**	14.67±0.33**
Fusarium oxisporum	16.3±0.61	23.4±0.86**	18.35±0.69**	14.4±0.51**	16.62±0.84**	10.8±0.048**	9.00±0.58*	12.33±0.88**
R.stolonifer	15.3±0.90	20.1±0.62**	16.9±0.51**	16.3±0.49**	13.45±0.51**	-	9.67±0.47*	10.2±0.33*

Standards: Ketoconazole; Activity index = ZOI of test sample/ZOI of standard. Mean±SE, * p< 0.05, ** p<0.01, All columns (ZOI) are compared with standard using Tukey test after ANOVA using ezanova software.

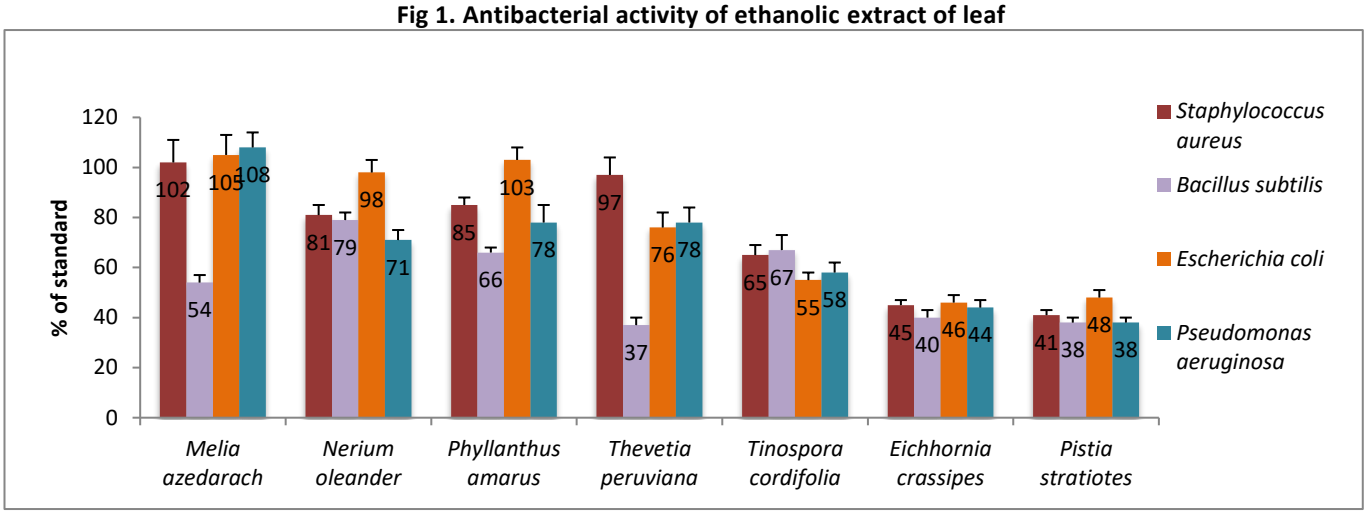
Table 4: Activity index of antibacterial bioassay of some medicinal plants in ethanol extract

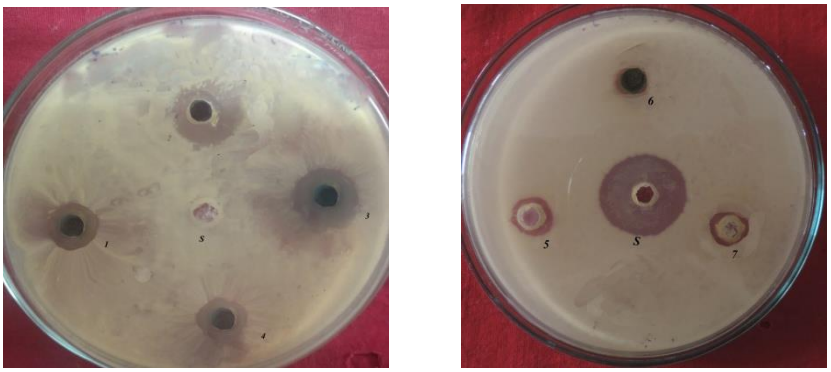
Pathogenic fungi	Activity Index (AI) (mm)							
	Standard	*Melia azedarach*	*Nerium oleander*	*Phyllanthus amarus*	*Thevetia peruviana*	*Tinospora cordifolia*	*Eichhornia crassipes*	*Pistia stratiotes*
Aspergillus niger	17.6±0.30	1.28	1.15	0.83	0.73	0.58	0.45	0.54
Aspergillus flavus	17.03±0.63	1.24	0.95	0.92	0.91	0.97	0.60	0.86
Fusarium oxisporum	16.3±0.61	1.43	1.12	0.88	1.01	0.66	0.55	0.75

R.stolonifer	15.3±0.90	1.31	1.10	1.06	0.87	00	0.63	0.65

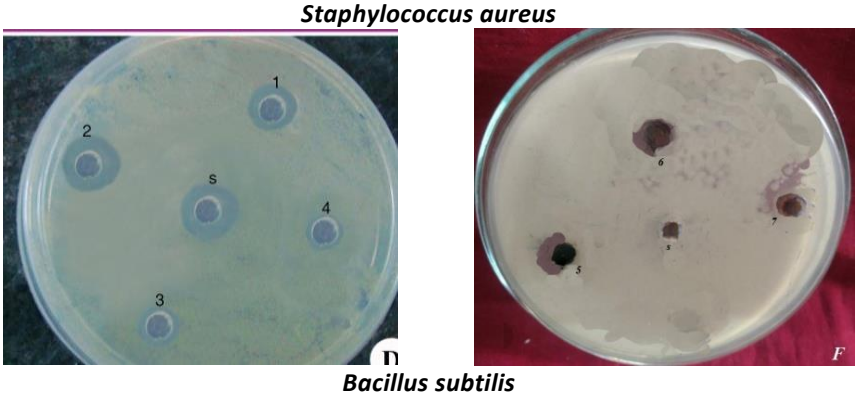
Activity index = ZOI of test sample/ ZOI of standard.

Fig 1. Antibacterial activity of ethanolic extract of leaf

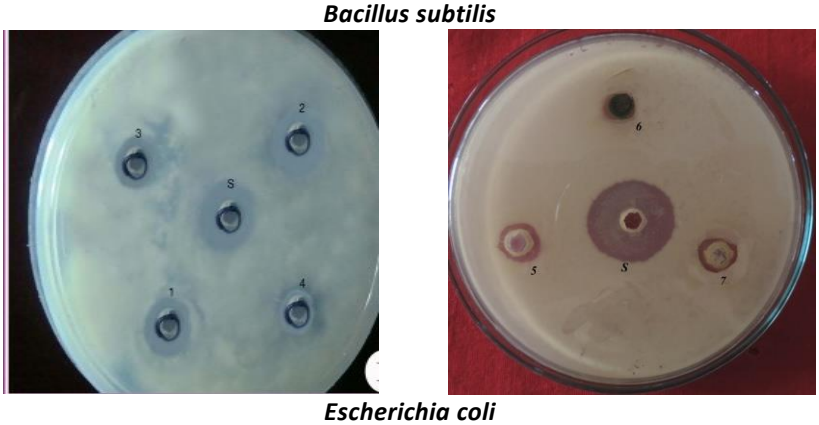




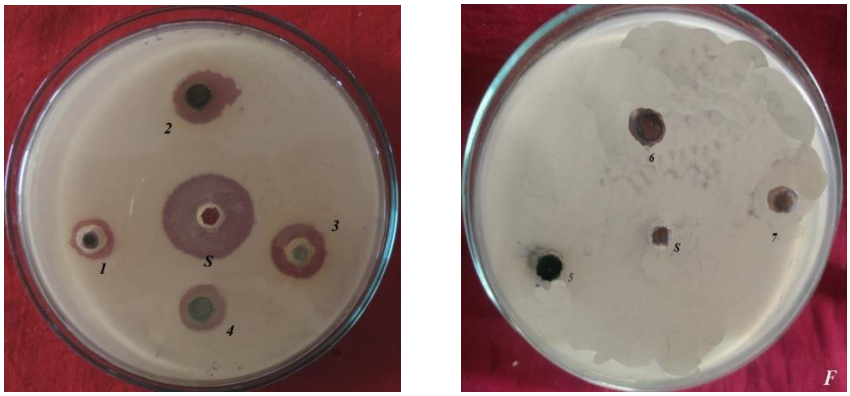
Staphylococcus aureus



Bacillus subtilis



Escherichia coli



Pseudomonas aeruginosa

Plate 1: Antibacterial activity of ethanol extract of leaves of different plants.

S- Standard 1. *Melia azedarach* 2. *Nerium oleander* 3. *Phyllanthus amarus* 4. *Thevetia peruviana* 5. *Tinospora cordifolia* 6. *Eichhornia crassipes* 7. *Pistia stratiotes*

For the antifungal activity, *Fusarium oxisporum* (23.4±0.86 mm) and *Aspergillus niger* (22.7±0.11 mm) showed efficient antifungal activity for ethanol plant extract. *A. flavus* (21.2±0.32 mm) and *R. stolonifer* (20.1±0.62 mm) showed proficient antifungal activity. *R. stolonifer* showed lowest inhibition zone with activity index 1.31 against all pathogenic fungal strains (Table 3& 4; Figure 2; Graph 2).

(ii) Nerium oleander

The ethanol extract of leaves of *N. oleander* inhibited the growth of all bacteria at p< 0.01. The antibacterial activity in ethanolic extract of *N. oleander* showed the highest inhibition zone 19.6±0.73 mm with activity index 0.79 in pathogen *Bacillus subtilis*. Ethanol extract of the same proved highly significantly toxic to the growth of *S. aureus* and at p< 0.01. Leaves of ethanol extract showed toxicity on *S. aureus*, *E. coli and P. aeruginosa* (AI 0.81, AI 0.98, and AI 0.71) than ETAC extract (Table 1 & 2; Figure 1).

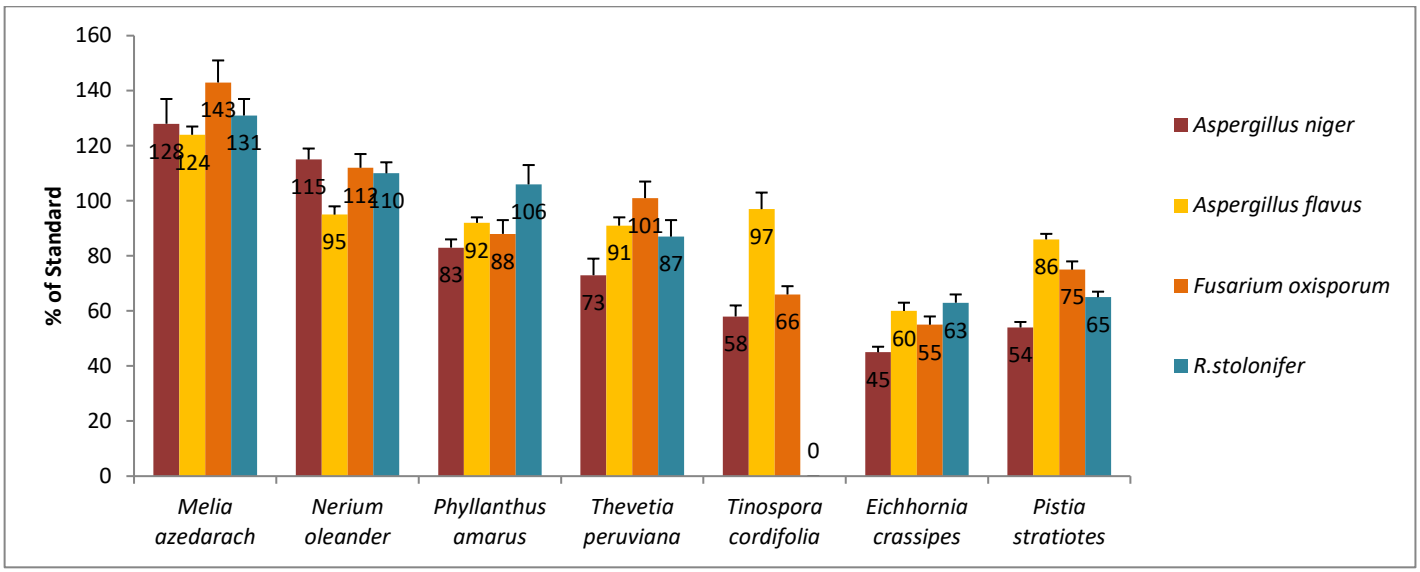
Antifungal activity of leaf of *N. oleander* exhibited activity index ranging from 0.95 to 1.15 for all fungi (Figure 2). The ethanol extract of leaves produced highest antifungal effect against *A. niger* (ZOI 19.6±0.51 mm; AI 1.15) significant at p< 0.01 (Table 3). Minimum toxicity was shown against *Aspergillus flavus* (ZOI 16.32±0.34 mm; AI 0.95) as compared to other fungal pathogens (Table 3& 4; Figure 2).

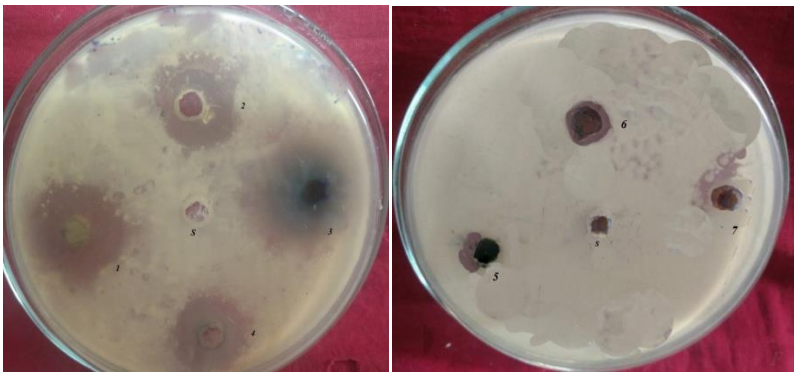
(iii)*Phyllanthus amarus* Schum. and Thonn.:

In the present investigation, ethanol extracts were found to have highest antibacterial activity against *S. aureus*, B. *subtilis,* and P. *aeroginosa,* respectively. In presence of ethanolic extract the maximum zone of inhibition was obtained (19.2±0.32 mm) in *E. coli,* (16.4±0.82 mm) in *B. subtilis*. Whereas antibacterial activity was obtained in *S. aureus* and *P. aeruginosa* with inhibition zone diameter of 16.3±0.91 mm and 15.8±0.45 mm, respectively (Table1& 2; Figure 1).

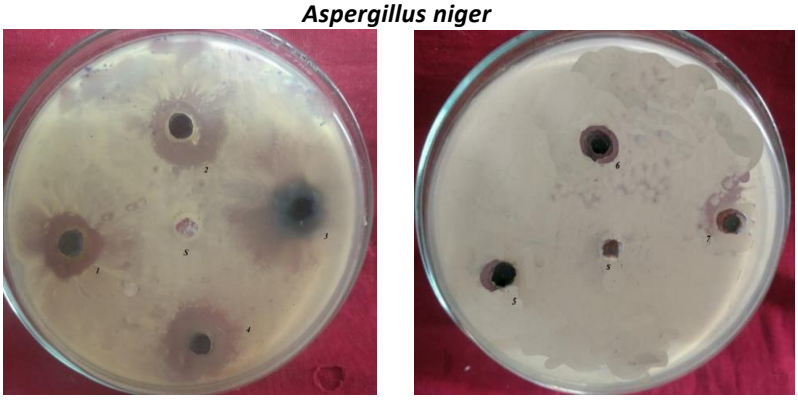
The antifungal activity was also studied for different extracts using 4 different fungal strains viz. *A. niger, A. flavus, R. stolonifer, F. oxisporum*. In the present study, for Ethanolic extract, maximum antifungal activity was observed against *A. niger* (16.3±0.75 mm) and *F. oxisporum* (14.4±0.51 mm), whereas Methanol extract was found to be much effective against *R. stolonifer* (16.3±0.49 mm) as compared to other tested microorganisms (Table 3 & 4; Figure 2).

Fig 2. Antifungal activity of ethanolic extract of leaf

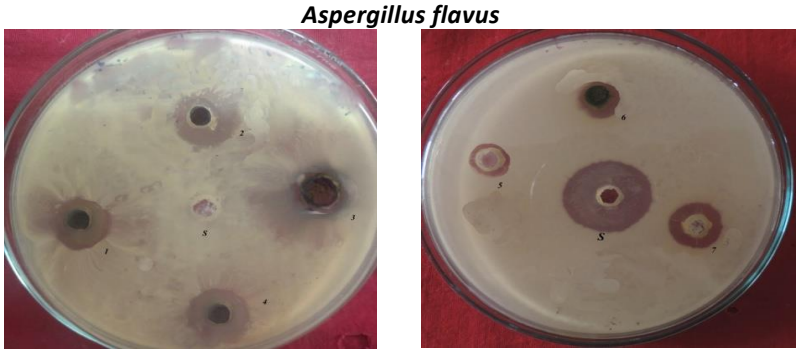




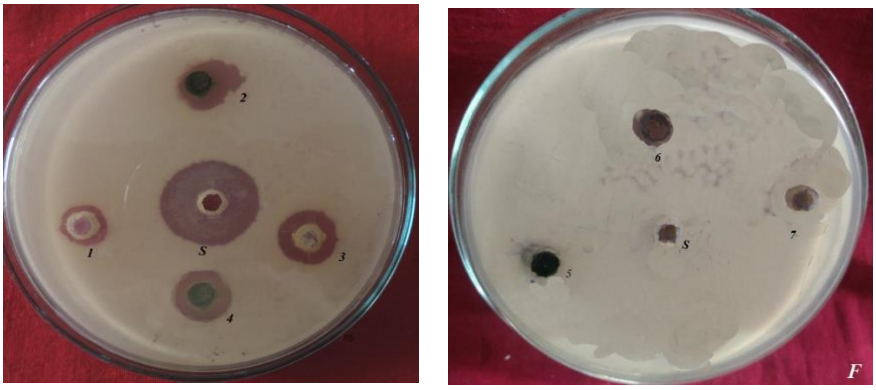
Aspergillus niger



Aspergillus flavus



Fusarium oxisporum



R. stolonifer

Plate 2: Antifungal activity of ethanol extract of leaves of different plants.

S- Standard 1. *Melia azedarach* 2. *Nerium oleander* 3. *Phyllanthus amarus* 4. *Thevetia peruviana* 5. *Tinospora cordifolia* 6. *Eichhornia crassipes* 7. *Pistia stratiotes*

4. Thevetia peruviana

Thevetia peruviana had significantly lethal effect on *S. aureus* in Ethanol extract of leaves (ZOI 18.6±0.23 mm; AI 0.97 at p< 0.01). Toxicity decreased against *Bacillus subtilis* (Table 1). Ethanolic extract of leaves was moderately toxic to the growth of *Escherichia coli and Pseudomonas aeruginosa with zone of inhibition* 14.3±0.57 and 7.3±0.35 mm and significant at p< 0.05. The activity index was ranged from 0.37 to 0.97 (Table1 & 2; Figure 1).

Antifungal activity of leaf of *Thevetia peruviana* was significantly toxic to the growth of all 4 fungi investigated in ethanol extracts (Table 3). The maximum toxicity was observed significant in leaves against growth of fungi *F. oxysporum* (ZOI 16.62±0.84 mm; AI 1.01) and reduced the growth of the latter. The leaf extract strongly decreased the growth of *Aspergillus flavus* with zone of inhibition 15.5±0.75 mm at p< 0.01. The moderate toxicity produced to the growth of *Aspergillus niger and R.stolonifer* with ZOI 15.5±0.75 MM and 13.45±0.51 mm respectively (Table 3& 4; Figure 2).

5. Tinospora cordifolia

The ethanolic extract of leaves of *T. cordifolia* inhibited the growth of all bacteria at p< 0.01. Nevertheless, this extract of the same proved highly significantly toxic to the growth of *B. subtilis* at p< 0.01. In this extract, leaves exhibited great toxicity (AI 0.67 at p< 0.01). Minimum toxicity on *S. aureus, E. coli and P. aeruginosa (AI 0.65, AI 0.55, and AI 0.58) than* ETAC extract (Table1& 2; Figure 1).

The toxic effect of ethanol extract leaf (AI 0.97) of *T. cordifolia* was higher than another fungal pathogen in *A. flavus*. The average zone of inhibition of leaves was observed 10.34±0.05mm and 10.8±0.048mm *in A. niger* and *F. oxysporum*, respectively. *A. niger* showed significant value at p< 0.05 and *F. oxysporum* showed at p< 0.01. The *T. cordifolia* showed no response in *R. stolonifer* (Table 3& 4; Figure 2).

6. Eichhornia crassipes

This plant showed average response to all bacteria than other plants. The maximum zone of inhibition was measured 10.00± 0.817mm (AI 0.40) In *B. subtilis* at p< 0.01. All values are significant at p< 0.01. The minimum zone of inhibition was observed 8.67±0.47mm and 8.67±0.47mm in *S. aureus* and *E. coli* at p< 0.01. The activity index was observed 0.45, 0.40, 0.46 and 0.44 of all four bacteria i.e., *S. aureus, B. subtilis, E. coli and P. aeruginosa*, respectively (Table1 & 2; Figure 1).

The toxic impact of *Eichhornia crassipes* leaf extract of ethanol was average relative to other fungal pathogens. The maximum zone of inhibition was observed 10.33±0.88 mm in *A. niger*. *A. niger* showed significant value at p< 0.01 (ZOI 8.00±0.81 mm) and *F. oxysporum* showed at p< 0.01 (ZOI 9.00±0.58 mm). The *E. crassipes* showed zone of inhibition 9.67±0.47 mm in *R. stolonifer* at p< 0.05 (Table 3& 4; Figure 2).

7. Pistia stratiotes

The maximum zone of inhibition of antibacterial activity was recorded significantly at p< 0.01 in *B. subtilis* and *E. coli* at p< 0.01. The minimum zone of inhibition was observed 8.00±0.00 mm and 8.33± 0.47 mm in *S. aureus* and *P. aeruginosa* at p< 0.05, respectively. The activity index was observed 0.41, 0.38, 0.48 and 0.38 of all four bacteria i.e *S. aureus, B. subtilis, S. aureus* and *P. aeruginosar,* respectively (Table1& 2; Figure 1).

The antifungal activity index was maximum recorded in case of *Aspergillus flavus* (AI-0.86) followed by *Fusarium oxisporum* (AI-0.75), *R. stolonifer* (AI-0.65) and *Aspergillus niger* (AI-0.54) significantly at p< 0.01. The maximum zone of inhibition was observed in *Aspergillus flavus* with zone of inhibition 15.5±0.75 mm and significantly minimum in *Aspergillus niger* (ZOI 12.9±0.66) (Table 3& 4; Figure 2).

DISCUSSION AND CONCLUSION

Plants used by folklore are valuable for modern medicine as they are direct sources of therapeutic agents. Plants have unlimited and untapped wealth of chemical compounds with very high drug potential, modern approaches are being employed for selecting the promising plants having medical ethnobotanical background and identifying the active principle. To evaluate the biological or pharmacological importance, various activities such as antibacterial, antifungal, antiviral, antitumor, anti-inflammatory, antipyretic and analgesic were tested to identify the active principles and their bio efficacy [17].

In the present study ethanolic extracts were checked for their antimicrobial activity, the leaf plant part of all plants *Eichhornia crassipes*, *Pistia stratiotes*, *Phyllanthus amarus*, *Melia azedarach*, *Nerium oleander*, *Thevetia peruviana* and *Tinospora cordifolia* showed significant results against bacteria and fungi. *B. subtilis* and *S. aureus* have been reported for their potent antibacterial and antifungal activity. The ethanol extract of all plants exhibited antibacterial activities, due to many biologically active compounds. Most effective activity against bacteria and fungi was shown in *Melia azedarach* and lowest zone of inhibition against *B. subtilis* and *Eichhornia crassipes* in bacteria and fungi, respectively (Graph 1&2). The increasing order of toxicity of medicinal plants against bacteria i.e., *M. azedarach* > *P. amarus* > *N. oleander* > *T. peruviana* > *T. cordifolia* > *E. crassipes* > *P. stratiotes*. The increasing order of toxicity against fungal species i.e., *M. azedarach* > *N. oleander* > *P. amarus* > *T. peruviana* > *T. cordifolia* > *P. stratiotes* > *E. crassipes*. Among the plants tested, *Eichhornia crassipes*, *Pistia stratiotes*, *Phyllanthus amarus*, *Melia azedarach*, *Nerium oleander*, *Thevetia peruviana* and *Tinospora cordifolia* was really encouraging and demonstrated strong inhibition of all bacterial and fungal pathogens tested. Numerous workers have found that garlic juice has significant antibacterial and antifungal properties and owing to these properties, garlic juice is very effective against different diseases [18, 19]. The findings of the present investigation specifically show that antibacterial and antifungal activity differs with plant species.

This research is a tentative assessment of plant antimicrobial activity. It suggests that many plants have the ability to manufacture novel metabolites. Plants with a wide range of action can help to discover new chemical groups of antibiotics that could serve as selective agents for the conservation of animal or human health and provide biochemical resources for the study of infectious diseases [20]. In this article, we explore the concept, current and future of medicinal plants, both as possible antimicrobial crude drugs and as a source of natural compounds that serve as new anti-infection agents. The present work therefore emphasises the use of extracted solvent of plants part againt bacteria and fungus pathogens.

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CONFLICTS OF INTEREST

We declare that we have no conflicts of interest among authors.

AUTHORS CONTRIBUTION

Dr. Neerja Singh and Dr. Tulika Tyagi experimented work and collected all evidence. Dr. Sudhir Kumar and Dr. Pooja Chaturvedi calculated data and perform computation work. These data and input were used to plan the manuscript and all authors discussed the methodology and conclusions and contributed to the final manuscript.

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