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Antimicrobial Activity of root extract of *Phyllanthus fraternus* Webster: An Ethnomedicinal plant

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Abstract

Phyllanthus fraternus Webster, a pan tropical weed originated from western India, belongs to family Euphorbiaceae and commonly known as "Bhumyamlaki". It is a medicinally very useful plant species which is frequently used by tribal of Gujarat to cure certain diseases like asthma, cough, diarrhea, diabetes, skin diseases and scabies. Current study is targeted the on the activity of antimicrobial metabolites from the root. Root were dried and extracted by methanol. This root extract of *Phyllanthus fraternus* was screened against eleven clinically important bacterial strains and three antifungal strain by disc diffusion method. Result showed maximum antibacterial activities against P.aeruginosa with zone of inhibition of 25 mm followed by S. typhiB with a zone of inhibition of 11 mm and minimum against *S. typhi* Awith zone of inhibition of 10 mm. Similarly the maximum antifungal activity was noted against *A. niger* (10 mm). Absolute methanol was used as control during the experiments. This indicates that antimicrobial activities may be due to presence of secondary metabolites. Hence, the plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities. As the antimicrobial work has been not carried out so far on *Phyllanthus fraternus*,

Keyword: *Phyllanthys fraternus*, root, extract, antimicrobial activities.

Introduction

The genus *Phyllanthus* belongs to the family Euphorbiaceae. The family has about 750 -1200 genera. The majority of genera are pan tropical weeds and They are distributed in all tropical and subtropical regions on the earth. *Phyllanthus fraturnus Webster* is one of the valuable medicinal plants and it has been used for the treatment of various ailments such as flu, dropsy, diabetes and jaundice. India has rich heritage of using medicinal plants as traditional medicines. Although hundreds of plant species have been tested for antimicrobial properties[1]. there is no report on antimicrobial properties of various plant parts like leaves, fruits and roots of *Phyllanthus fraternus* Webster against the bacterial and fungal microorganisms.

The world Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies among 80% of the world population[2]. Over 50% of all modern clinical drugs are of natural product origin[3]. The potential of higher plants as a source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction is submitted to biological or pharmacological screening[4].

The present study is aimed to carry out antimicrobial activity of the root extract against eleven clinically important bacterial strains and three fungal strain by using agar disc diffusion method.

Material and methods

Plant material (Figure-1): The roots of *Phyllanthus fraternus* Webster were collected from Botanical garden of S. K. Pharmaceutical college of education and research; Ganpat University; Ganpat vidyanagar; Kherwa, North Gujarat. The plant was identified by using the Flora of Gujarat by G. L. Shah (1978). The plant is annual having the length of 20-50 cm. It produces pale greenish -yellow colored axillary flowers. The seeds are trigonous. This plant is used by tribals of Gujarat to cure certain diseases like asthama, cough, diarrohea and scabies.

Extraction of plant root: The roots of the plants were washed under running tap water and than by distilled water to remove soil particles and dirt. The roots were dried at room temperature in the laboratory. The dried roots were crushed to make powder using grinding machine. Root powder was stored at 4^0C in tight air container bottle.

The extraction of roots was done by methanol using Soxhlet apparatus. The solvent was evaporated by using rotary evaporator at 80^0C temperature and the extract obtained was cooled and dried under vacuum.

Figure-1
Root of *Phyllanthus fraternus* plant

Bacterial and fungal strains used: Microbial strains *Escherichia coli, Bacillus megaterium, Bacillus cereus, Bacillus subtilis, Cornynebacterium glutamicum, Staphylococcus aureus, Salmonella typhi, Salmonella typhi A, Salmonella typhi B, Pseudomonas aeruginosa, Proteus vulgaris,* and fungal strain *Aspergillus* niger, penicillium chryosogenus and candida were obtained from MUIS ,Ganpat University, Mehsana and M. G. Science Institute , Ahmedabad (Gujarat).

Antimicrobial assay for disc diffusion method: Antimicrobial assay of solvent extracts were performed by Disc diffusion method. For bacteria Nutrient broth and Fungal Potato dextrose broth, pH was adjusted to 7.2 and 7.0. respectively. Suspensions of the different strains of the bacteria were added to sterile Nutrient broth and that of fungal strains were added to sterile Potato dextrose broth in separate petridishes . Bacterial and fungal strains were swabbed separately on the Nutrient agar plate and Potato dextrose agar plate respectively aseptically. The sterile disc, 5mm in diameter, is saturated with 10µL to 50 µL /10ml test culture of methanolic extracts separately. The discs with absolute methanol is used as control. The bacterial plates were incubated at 37^0C for 24 hr while fungal plates were incubated at 28^0C for 24-48 hr . The sterile impregnated discs with root extract were placed on the agar surface with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar surface.

After the incubation, the size of the inhibition zone was measured. Antimicrobial activities were determined by measuring the diameter of the zone of inhibition surrounding microbial growth. For each strain, control performed that comprised pure solvent instead of the root extract[5].

Results and Discussion

In the present investigation antimicrobial activities of methanol extracts of root of *Phyllanthus fraternus,* in terms of zone of inhibition was presented in table-1.

Table-1
Zone of inhibitory activity (in millimeter) of methanol root extracts against microorganisms

Sl. no.	Organism	Zone of inhibition (mm)				
		10	**20**	**30**	**40**	**50**
1.	*Escherichia coli*	10	No	No	No	No
2.	*Bacillus megaterium*	16	No	10	11	No
3.	*Bacillus cereus*	11	10	14	11	13
4.	*Bacillus subtilis*	19	12	10	10	12
5.	*Cornynebacterium glutamicum*	11	10	12	10	10
6.	*Stephylococcus aureus*	17	10	12	13	10
7.	*Salmonella typhi*	21	12	11	10	13
8.	*Salmonella typhi A*	11	12	10	13	11
9.	*Salmonella typhi B*	11	11	11	11	10
10.	*Pseudomonas aeruginosa*	25	20	23	22	20
11.	*Proteus vulgaris*	19	10	11	10	11
12.	*Aspergillus niger*	No	No	No	10	No
13.	*P.chrysogenus*	11	12	13	11	11
14.	*Candida*	10	11	11	11	11

The results obtained in the present study revealed that tested root extracts of *Phyllanthus fraternus* possess potential antibacterial activity against all 11 bacterial strains and 3 fungal strain. When tested by disc diffusion method , the methanol root extract showed significant activity where inhibition zone ranged between 10 mm and 25 mm against all the microorganisms. The highest antibacterial activity of 25 mm in *Pseudomonas aeroginosa* and least activity of 11 mm in *Salmonella typhi A* were recorded in methanol extract Similarly, *Bacillus megaterium and Salmonella typhi* revealed maximum activity of 16 mm and 21 mm respectively in methanol extract. It is also examined from the present data that methanolic root extract shows significant higher rate of sensitivity against fungal strain *Aspergillus niger* and *Penicillium chryosogenus*.

Discussion: The medicinal properties of plants are due to the presence of different complex chemical substances which are exclusively accumulated in different parts of the plants and produce marked healing action on human body[6,7]. The most important of these agents are alkaloids, flavanoids and tannins[8,9]. These compounds have been associated with antimicrobial effects in various studies using plant extracts[10].

Antibacterial compounds pf plant origin have enormous therapeutical potential as they can serve the purpose without side effects that are often associated with synthetic antimicrobials[11,12]. In the present work the methanol root extracts of *Phyllanthus fraturnus* were used for antimicrobial activity against 11 bacterial and 3 fungal strains.

The test organisms used in the study are associated with various forms of human infections. Apart from *Salmonella typhi*

infection, *Salmonella paratyphi* A and B also widely persist in Indian population[13]. In the present study methanolic root extracts of *Phyllanthus fraternus* showed considerable inhibitory activity against both enteric isolates of *Salmonella typhi* and *Salmonella paratyphi*[12] have also shown that reports of such similar work on enteric *Salmonella paratyphi* (A and B).

Conclusion

From the results of present investigation it is reported that root extract prepared in methanol shows significant higher antimicrobial activity against all test microorganisms revealing inhibition zones between 10 mm and 25 mm The present study ascertains the value of solvents used in the drug preparation, which could be of considerable interest to the development of new drugs. The fact that the root extract of *Phyllanthus fraternus* was active against all the tested microorganisms is also an indication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms. As there is no report on antimicrobial activity of root extract of *Phyllanthus fraternus,* further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

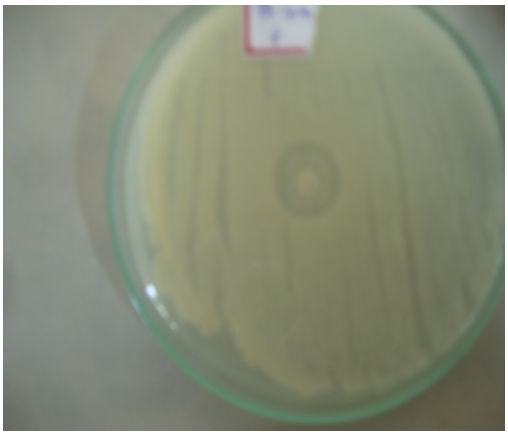
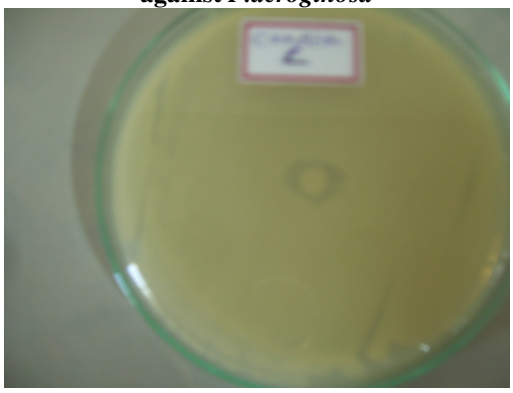


Figure-2

Zone of inhibition A: Methanol root extract and B: Control against *P.aeroginosa*



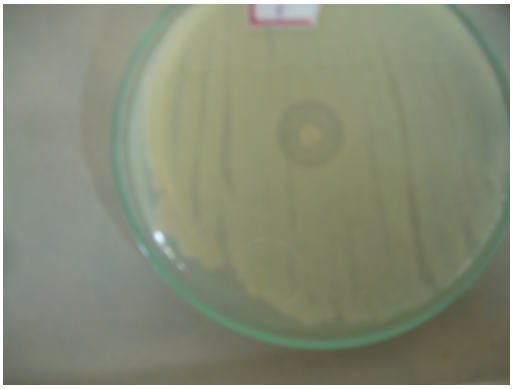


Figure-3
Zone of inhibition A & B: Methanol root extract against *c.glutamicum and B.subtillus*

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