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ANTIBACTERIAL ACTIVITY OF FRUIT EXTRACT OF *PHYLLANTHUS FRATERNUS* WEBSTER: AN ETHNOMEDICINAL PLANT

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ABSTRACT

The antimicrobial activity of plant extract has been recognized for many years. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found *in vitro* to have antimicrobial properties. These herbal products are useful to discover new agents. *Phyllanthus fraternus* Webster, belonging to family Euphorbiaceae is a medicinally very useful plant species used by tribal of Gujarat to cure certain diseases like asthma, cough, diarrhoea, diabetes, skin diseases and scabies. In the present study Ethanolic fruit extracts (1000 mg/10 ml) of *Phyllanthus fraternus* were screened against bacterial strains such as *E. coli*, *B. megaterium*, *B. cereus*, *B. subtilis*, *C. glutenicum*, *S. aureus*, *S. typhi*, *S.typhi A*, *S. typhi B*, *P. aeruginosa* and *P. vulgeris* by disc diffusion method. The result showed maximum antibacterial activities against *B. cereus* with zone of inhibition of 28 mm followed by *C. glutenicum* with a zone of inhibition of 27 mm and *S. typhi* A with zone of inhibition of 25 mm. Absolute ethanol was used as control during the experiments. This indicates that antimicrobial activities may be due to the 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. So far antibacterial work has been not carried out on *Phyllanthus fraternus* hence, the attempts were made to study the same.

KEYWORD: *S.typhi*, fruit extract, *P. fraternus.*

INTRODUCTION

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents and intravenous catheters. (Gonzalez *et al.*,1996). In the last three decades though pharmacological industries have produced many new antibiotics, microbial resistance to these has increased a lot because of genetic ability of bacteria to acquire and transmit the resistance against therapeutic agents. (Mohanta *et al.*, 2006).

Plant as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. The World Health Organisation estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies among 80% of the world population (Anonymous, 1993). Over 50% of all modern clinical drugs are of natural product origin (Baker *et al.*, 1995). 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 (Vashist and Jindal, 2012).

India has rich heritage of using medicinal plants as traditional medicines. Although hundreds of plant species have been tested for antimicrobial properties (Uzun *et al.*, 2002; Ates *et al.*, 2003; Kirbag *et al.*, 2005; Nair, 2005; Kumar *et al.*, 2006, Doughari *et al.*, 2008, Kirbag *et al.*, 2009; Dash *et al.*, 2011; Dubey *et al.*, 2012, Bashir *et al.*, 2012 and Vashist and Jindal, 2012), there is no report on antimicrobial properties of various plant parts like leaves, fruits and roots of *Phyllanthus fraternus* Webster against the various bacterial strains. The present study is aimed to carry out the preliminary phytochemical analysis and to screen *in vitro* antimicrobial activity of the fruit extract against thirteen clinically important bacterial strains by using agar disc diffusion method.

MATERIAL AND METHODS

Plant material (Fig. 1)

The fruits 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.

Fig. 1 A twig of *Phyllanthus fraternus.*

Extraction of Fruit

The fresh fruit collected from the plant were washed under running tap water followed by distilled water to remove soil particles and dirt. The fruit were air dried at room temperature in the laboratory. The dried fruit were crushed to powdered using grinding machine. Fruit powder was stored at 4^0C in tight air container bottle.

The extraction of fruits was done by Ethanol 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.

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the phytoconstituents as described by Harbone (1973), Sofowara (1979) and Trease and Evans (1983).

Determination of constitute by HPTLC

HPTLC was performed on 20cm X 10cm aluminum plates, pre-coated with 0-2 mm layer of silica gel $60F_{254}$ (Merck, Germany). The samples were applied in band with a Linomat 5 applicator, CAMAG (Switzerland) equipped with a 100 µL syringe. Plates were developed vertically in a CAMAG twin trough chamber previously saturated with Toluene-chloroform-ethanol (4:4:1) mobile phase vapour for 20 minutes at room temperature.

Bacterial strains used

Total 13 bacterial strain *Escherichia coli, Bacillus megaterium, Bacillus cereus, Bacillus subtilis, Cornynebacterium glutamicum, Staphylococcus aureus, Salmonella typhi, Salmonella typhi A, Salmonella typhi B, Pseudomonas aeruginosa, Proteus vulgaris* were obtained from MUIS, Ganpat University, Mehsana and M. G. Science Institute, Ahmedabad (Gujarat).

Antibacterial activity for disc diffusion method

The sterile disc, 5mm in diameter, is saturated at concentration of 1000 mg /10 ml and various amount of extract 10 µl to 50 µl ethanolic fruit extracts against various bacterial culture The disc with absolute ethanol used as control. The bacterial plates were incubated at 37^0C for 24 hr. The sterile impregnated discs with plant extracts 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. Antibacterial activities were determined by measuring the diameter of the zone of inhibition surrounding microbial growth. For each strain, controls were included that comprised pure solvents instead of the extract (Parekh and Chanda, 2007).

RESULTS

In the present investigation, preliminary screening of ethanolic fruit extract of *Phyllanthus fraternus* showed the presence of alkaloids, tannins, terpenoids and flavanoids. (Table- 1).

Table: 1 Analysis of phytochemical constitute in the fruit extract of *Phyllanthus fraternus*.

Phytochemical constituents	Ethanolic fruit extract
Alkaloids	**Present**
Tannins	**Present**
Saponins	Absent
Phlobatannins	Absent
Flavanoids	**Present**
Terpenoids	**Present**
Glycosides	Absent
Steroids	Absent

Determination of constitutes by HPTLC showed that under 366 nm (Fig. 2) while after 1 hr at 110^0 C treatment under 366 nm orange brown bands were observed which correspond alkaloid compounds (Fig. 2).

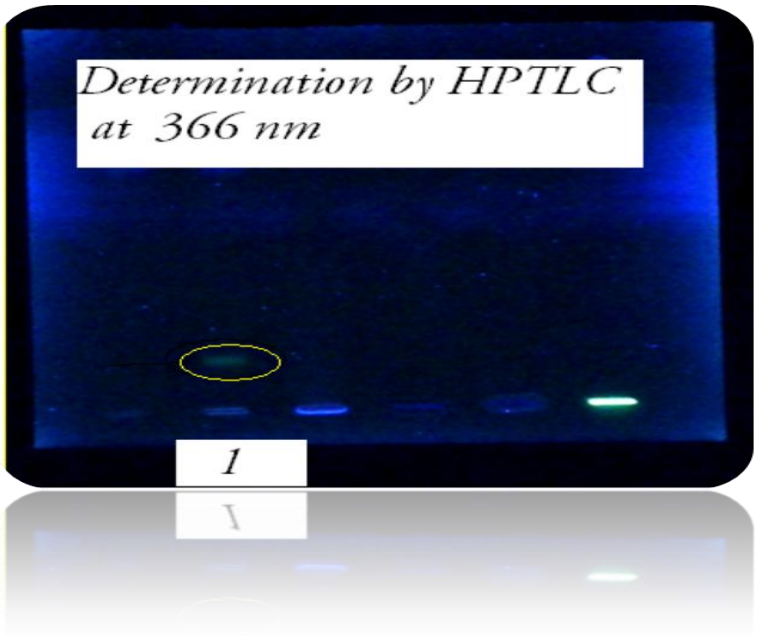

Fig 2: HPTLC at 366 nm.

Table 2: Zone of inhibitory activity (in millimeter) of ethanol Fruit extracts against various bacterial strain.

Sr.no.	Organism	Ethanol Fruit extract Zone of inhibition (mm)				
		10ul	**20ul**	**30 ul**	**40ul**	**50 ul**
1.	*Escherichia coli*	12	18	21	22	23
2.	*Bacillus megaterium*	26	20	25	26	22
3.	***Bacillus cereus***	**14**	**14**	**18**	**22**	**28**
4.	*Bacillus subtilis*	14	16	19	19	22
5.	***Cornynebacterium glutamicum***	**12**	**13**	**13**	**16**	**27**
6.	*Stephylococcus aureus*	0	0	0	0	0
8.	*Salmonella typhi*	13	15	16	18	20
9.	***Salmonella typhi A***	**12**	**15**	**16**	**19**	**25**
10.	*Salmonella typhi B*	11	14	15	18	24
11.	*Pseudomonas aeruginosa*	17	20	20	16	23
12.	*Proteus vulgaris*	15	18	19	20	22
13.	*Bacillus amyloliqif.*	0	0	0	0	24

The *in vitro* antibacterial of ethanol fruit extract of *Phyllanthus fraternus*, in terms of zone of inhibition was presented in Table 2.

The results obtained in the present study revealed that tested fruit extracts of *Phyllanthus fraternus* possess potential antibacterial activity against all 13 bacterial strains. When tested by disc diffusion method, the ethanol fruit extract showed significant activity where inhibition zone ranged between 12 mm and 28 mm against all bacterial strain.

The highest antibacterial activity of 28 mm in *Bacillus cereus* and least activity of 22 mm in *Proteus vulgaris* were recorded in ethanol extract (Plate:1, Figs. 1,2 & 3) Similarly, *Bacillus cereus, Cornynebacterium glutamicum and Salmonella typhi A* revealed respective maximum activity of 28 mm,27 mm and 25 mm each in ethanol extract. It is also examined from the present data that ethanolic fruit extract shows significant higher rate of sensitivity (28 mm) against other strain.

DISCUSSION

Our preliminary phytochemical screening revealed the presence of alkaloids, tannins, terpenoids and Flavanoid in *Phyllanthus fraturnus*. The medicinal properties of plants are due to the presence of different complex chemical substances as secondary metabolites, which are exclusively accumulated in different parts of the plants and produce marked healing action on human body (Bashir *et al.,* 2012). The most important of these agents are alkaloids, flavanoids and tannins (Edeoga *et al.,* 2005). These compounds have been associated with antimicrobial effects in various studies using plant extracts (Abo *et al.,* 1999; Nweze *et al.,* 2004 and Nwaogu *et al.,* 2007).

Plant based antibacterial compounds have enormous therapeutical potential as they can serve the purpose without side effects that are often associated with synthetic antimicrobials (Sukanya et al., 2009). In the present work ethanol fruit extracts of *Phyllanthus fraturnus* were used for preliminary screening for antibacterial activity against 13 bacterial 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 (Prasannabalaji *et al.,* 2012). In the present study ethanolic fruit extract of *Phyllanthus fraternus* showed considerable inhibitory activity against both enteric isolates of *Salmonella typhi* and *Cornynebacterium glutamicum.* Fruit extracts of *Phyllanthus fraturnus* also show high activity against other strain with zone of inhibition of 28 mm in ethanol extract, the inhibition zone of 12 mm or more is considered as high antimicrobial activity (Veeramuthu *et al.*, 2006). and this indicates that the antibacterial activities also vary with the

solvents used. This tend to show that active ingredients of the fruit are better extracted with ethanol. Eloff (1998), Ahmad *et al.*, (1998), Lin *et al.*, (1999), Cowan (1999) and Bashir *et al.*,(2012) also found that ethanol is more efficient than other solvents in extracting phytochemicals from plant materials. 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 fruit 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 antibacterial activity of fruit extract of *Phyllanthus fraternus,* further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

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