

# Antibacterial and Antifungal Potentiality of Leaf Extract of *Phyllanthus Fraternus* Webster: An Ethnomedicinal Plant

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Received March 04, 2014; Revised March 28, 2014; Accepted March 31 2014

**Abstract** *Phyllanthus fraternus* Webster is a medicinally very useful plant species used by tribal of Gujarat, India to cure certain diseases like asthma, cough, diarrhoea and scabies. Methanolic and ethanolic leaf extracts of the plant leaf were evaluated against eleven clinically important bacterial strains such as *E. coli*, *B. megaterium*, *B. cereus*, *B. subtilis*, *C. glutamicum*, *S. aureus*, *S. typhi*, *S. typhi A*, *S. typhi B*, *P. aeruginosa* and *P. vulgaris* and one fungal strain namely *A. niger* by disc diffusion method. Result showed maximum antibacterial activities against *C. glutamicum* with zone of inhibition of 40 mm and minimum against *S. typhi A* with zone of inhibition of 15 mm in methanol extract. In ethanol extract maximum antibacterial activities were reported in *Bacillus cereus* with zone of inhibition of 21 mm and minimum against *Salmonella typhi A* (10 mm). The maximum antifungal activity was noted against *A. niger* (40 mm). Our preliminary phytochemical analysis of leaf extract using Methanol as solvent confirmed the presence of alkaloids, tannin, saponin, terpenoid and steroids. 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.

**Keywords:** alkaloid, bioactive, natural product

**Cite This Article:** Kavit Mehta, Patel B.N, and Jain B. K, "Antibacterial and Antifungal Potentiality of Leaf Extract of *Phyllanthus Fraternus* Webster: An Ethnomedicinal Plant." *American Journal of Microbiological Research*, vol. 2, no. 2 (2014): 74-79. doi: 10.12691/ajmr-2-2-6.

produces pale greenish -yellow colored axillary flowers. The seeds are trigonous.

## 1. Introduction

India has rich heritage of using medicinal plants in traditional medicines. Although hundred 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 plants parts like leaves, fruit, root of *Phyllanthus fraternus* Webster against the bacterial and fungal microorganisms. The present study is aimed to carry out the preliminary phytochemical analysis and to screen *in vitro* antimicrobial activity of the leaf extract against eleven clinically important bacterial strains and one fungal strain by using agar disc diffusion method.

## 2. Material and Methods

The leaves of plant of *Phyllanthus fraternus* Webster were collected from Botanical garden of Ganpat University; Ganpat vidyanagar; Kherwa, North Gujarat. The plant is annual having the length of 20-50 cm. It



Figure 1. A twig of *Phyllanthus fraternus*

### 2.1. Extraction of Plant Leaves

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

### 2.2. 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).

### 2.3. Determination of Constitutes by TLC

For TLC analysis plates of 10X10 cm size with aluminum support silica gel 60F<sub>254</sub>, (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Plates were impregnated by dipping into 4 % solution of sodium acetate in methanol –water (3:2) for 5 second followed by drying at room temperature for 1 hr .Glass capillaries were used to spot the sample Plates were developed vertically in a CAMAG twin trough chamber previously saturated with Toluene-ethyl acetate-diethylamine (7:2:1) mobile phase for 20 min. After the run, plates were dried and dragndorff reagent was sprayed at room temperature for detection of active compounds.

### 2.4. 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<sub>254</sub> (Merck, Germany). Samples were applied in band with a Linomat 5 applicator, CAMAG (Switzerland) equipped with a 100 uL 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.

The CAMAG TLC Scanner, controlled by winCATS software was used for densitometry analysis. Densitometry analysis was carried out under UV (at 256,366 and 540 nm) using CAMAG TLC Scanner and spraying with solution followed by heating at 110°C for 1 hr under observation.

### 2.5. 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, Candida albican and fungal strain Aspergillus niger and Candida albicans were obtained from MUIS, Ganpat university, Mehsana and M. G. Science institute, Ahmedabad (Gujarat).

### 2.6. Antimicrobial Assay for Disc Diffusion Method

Antimicrobial assay of solvent extracts were performed by Disc diffusion method. For bacteria Nutrient broth and for fungi Potato dextrose broth were used and pH was adjusted to 7.2 and 7.0 respectively with 5 M sodium hydroxide. Bacteria and fungi were swab separately on the Nutrient agar plate and Potato dextrose agar plate respectively aseptically. The sterile disc, 5mm in diameter, is saturated at concentration of 10ul/10ml test culture methanolic extract and ethanolic extract. Disc with absolute methanol and ethanol is used as a control. The bacterial plates were incubated at 37°C for 24 hr while fungal plates were incubated at 28°C for 24-48 hr. The sterile impregnated disc with plant 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.

Antibacterial and antifungal activity was 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).

Zone of diameter was showing no growth of the given organism has been reported as MIC of the test culture against Bacteria and fungi.

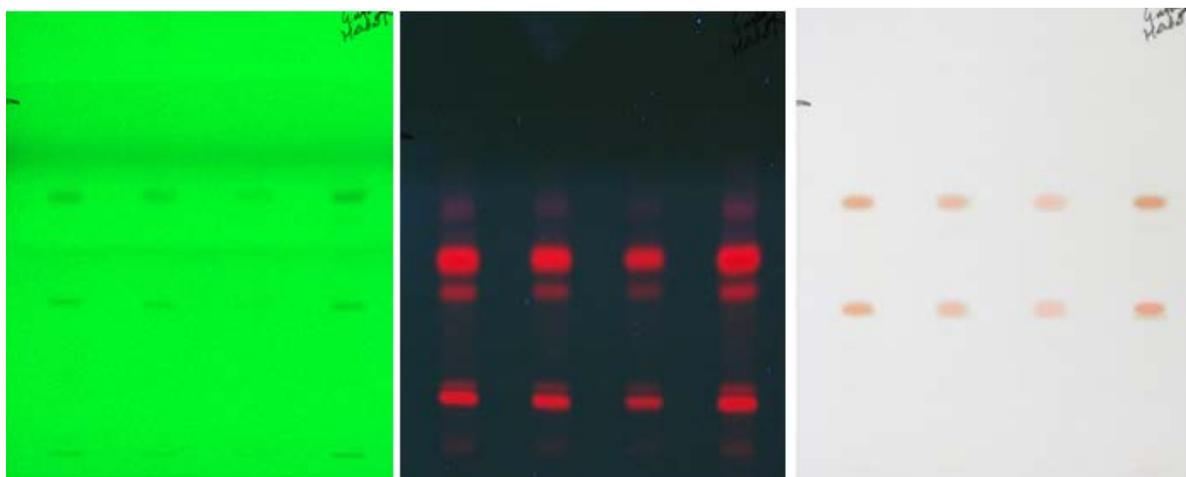
### 3. Results

**Table 1. Analysis of phytochemicals in the leaf extract of *Phyllanthus fraternus***

Phytochemical constituents ( in methanolic leaf extract)	
Alkaloids	Present
Tannins	Present
Saponins	Absent
Phlobatannins	Absent
Flavanoids	Absent
Terpenoids	Present
Glycosides	Absent
Steroids	Present

Reference: Mehta Kavita, Patel, B.N. Jain, B. K., Research Journal of Recent sciences, 2013, 2 : 12-15

Determination of constitutes by HPTLC showed that under 256 nm and 366 nm (Figure 2A and Figure 2B) only chlorophyll was observed while after 1 hr at 110°C treatment under 540 nm orange brown bands were observed which correspond alkaloid compounds (Figure 2C).



**Figure 2.** A: HPTLC at 256 nm, B: HPTLC at 366 nm, C: HPTLC at 540 nm

The *in vitro* antibacterial and antifungal activities of methanol and ethanol extracts of leaves of *Phyllanthus fraternus*, in terms of zone of inhibition was presented in Table 2.

**Table 2. Zone of inhibitory activity (in millimeter) of methanol and ethanol leaf extracts against microorganisms**

Sl.no.	Organism	Zone of inhibition	
		Methanol extract	Ethanol extract
1.	<i>Escherichia coli</i>	25 mm	13 mm
2.	<i>Bacillus megaterium</i>	33 mm	16mm
3.	<i>Bacillus cereus</i>	32 mm	21mm
4.	<i>Bacillus subtilis</i>	31 mm	15 mm
5.	<i>Corynebacterium glutamicum</i>	40 mm	12 mm
6.	<i>Stephylococcus aureus</i>	39 mm	12 mm
7.	<i>Salmonella typhi</i>	19 mm	15 mm
8.	<i>Salmonella typhi A</i>	15 mm	10 mm
9.	<i>Salmonella typhi B</i>	20 mm	16 mm
10.	<i>Pseudomonas aeruginosa</i>	18 mm	11 mm
11.	<i>Proteus vulgaris</i>	16 mm	15 mm
12.	<i>Aspergillus niger</i>	40 mm	No inhibition

## 4. Discussion

Our preliminary phytochemical screening revealed the presence of alkaloids, tannins, terpenoids and steroids in *Phyllanthus fraternus*. 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; Newze *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).

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 methanolic and ethanolic leaf extracts of *Phyllanthus fraternus* showed considerable inhibitory activity against both enteric isolates of *Salmonella typhi* and *Salmonella paratyphi*. Prasannabalaji *et al.*, (2012) have also shown that reports of such similar work on enteric *Salmonella paratyphi* (A and B) from scientific group is very minimal.

*E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immunodeficient patients (Doughari *et al.*, 2008). Leaf extract of *Phyllanthus fraternus* also shows high activity against *E. coli* with zone of inhibition of 25 mm in methanol extract but poor in ethanol extract (13 mm) as the inhibition zone

of 14 mm or more considered as high antimicrobial activity (Veeramuthu *et al.*, 2006).

From the results of present investigation it is reported that leaf extract prepared in methanol shows significant higher antimicrobial activity against all test microorganisms revealing inhibition zones between 15 mm and 40 mm as compared to that reported in ethanol leaf extract (inhibition zone ranges between 11mm and 16 mm) indicating that the antimicrobial activities also vary with the solvents used. This tend to show that active ingredients of the leaf are better extracted with methanol than ethanol. Eloff (1998), Ahmad *et al.*, (1998), Lin *et al.*, (1999), Cowan (1999) and Bashir *et al.*, (2012) also found that methanol 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 leaf 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 leaf extract of *Phyllanthus fraternus*, further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

## Acknowledgement

One of the authors, B. K. Jain is thankful to University Grants Commission, New Delhi, India for providing travel grants.

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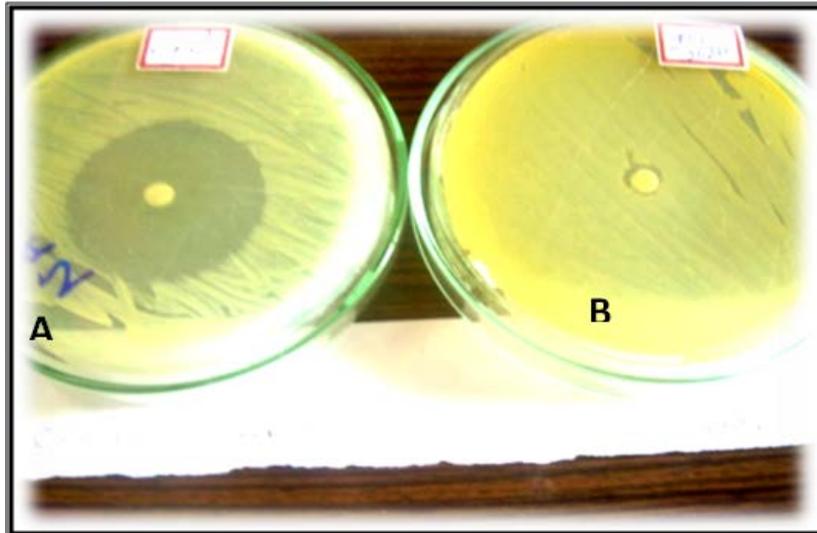


Figure 3. Zone of inhibition A: Methanol leaf extract and B: Control against *C. glutamicum*

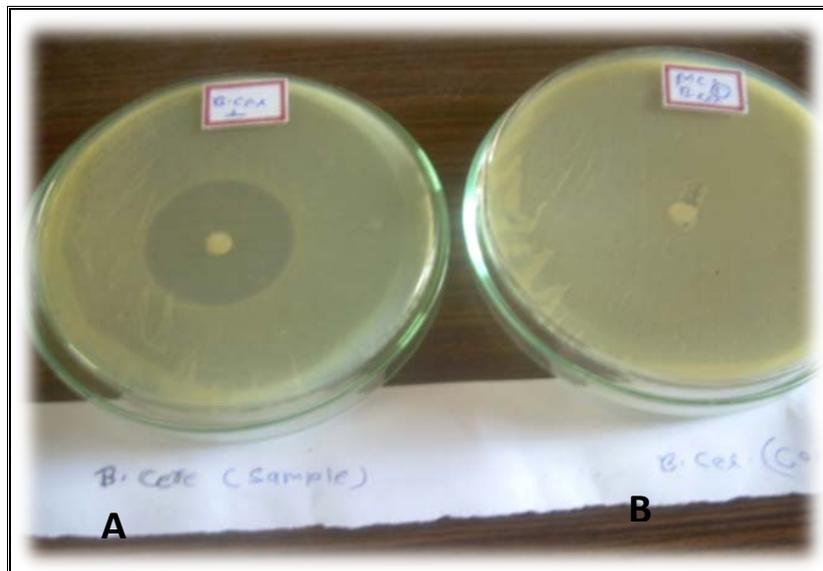


Figure 4. Zone of inhibition A: Methanol leaf extract and B: Control against *B. cereus*

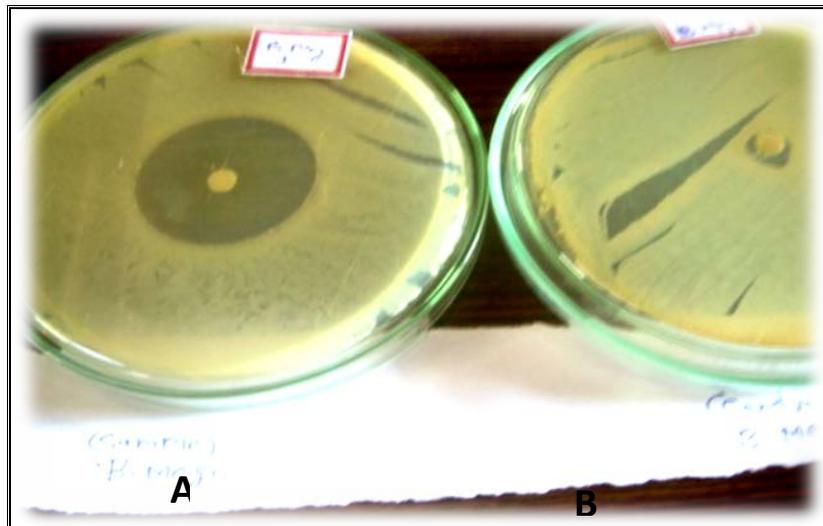


Figure 5. Zone of inhibition A: Methanol leaf extract and B: Control against *B. megaterium*

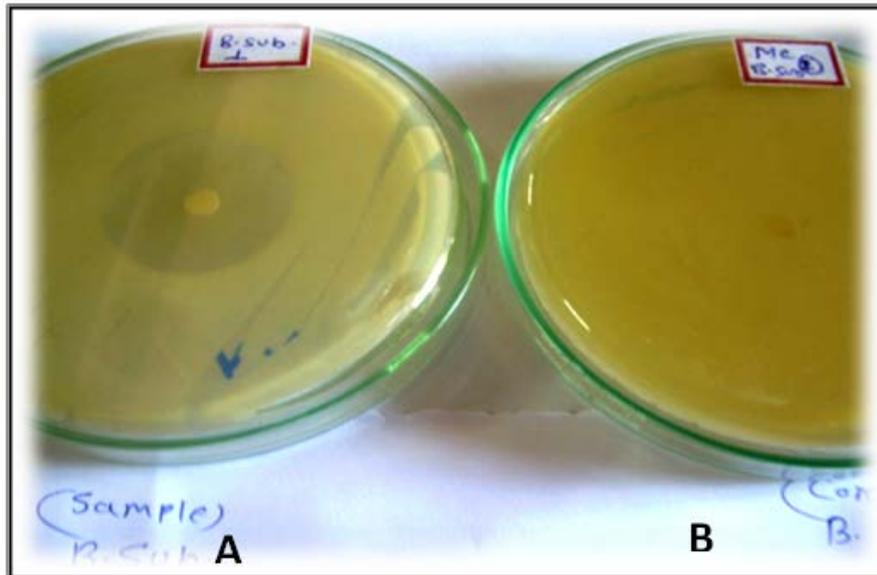


Figure 6. Zone of inhibition A: Methanol leaf extract and B: Control against *B. Subtilis*

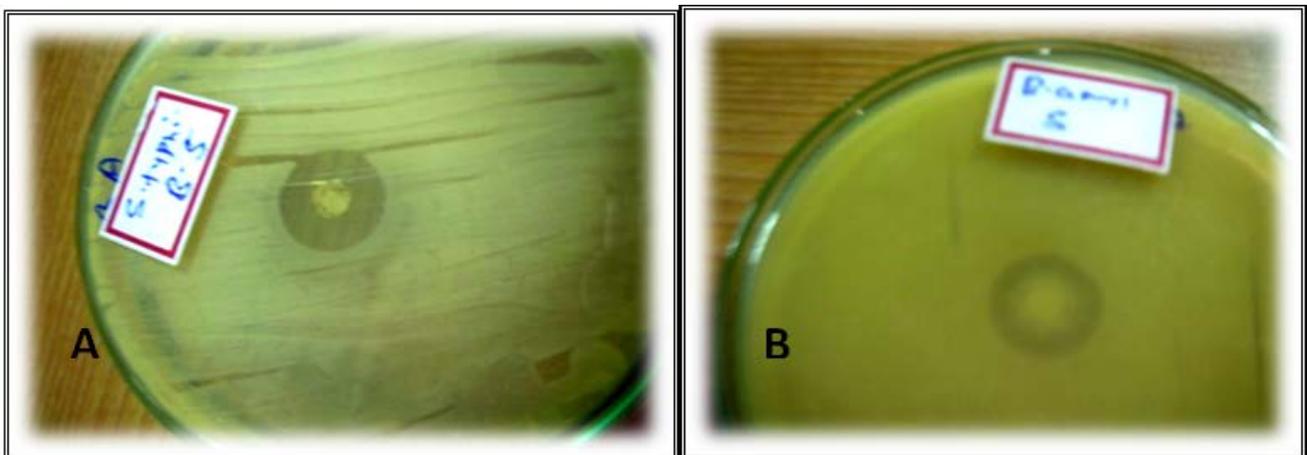


Figure 7. Zone of inhibition, Ethanol leaf extract against A: *B. amyloliquefaciens* and B: *S. typhi B*

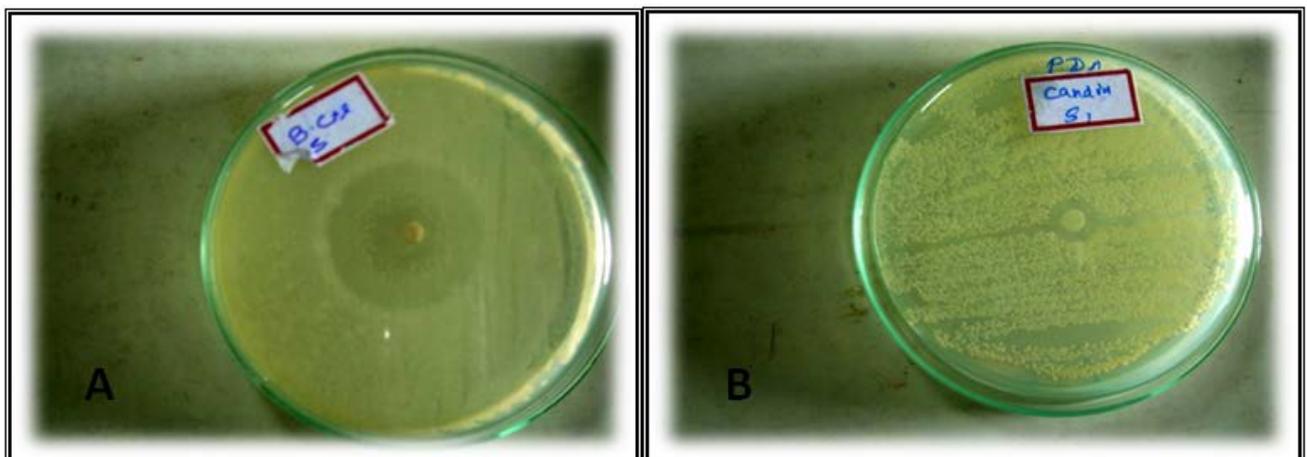


Figure 8. Zone of inhibition Ethanol leaf extract against A: *B. cereus* and B: *C. albicans*

## Plate - 2

### Review Comment Answer

1. Please mention whether the strain

Yes all the strain used were standard strain obtained from MTCC and some collected from Gujarat university microbiology laboratory detail is given below:

Organism	MTCC
<i>Escherichia coli</i>	4296
<i>Bacillus megaterium</i>	4911
<i>Bacillus cereus</i>	6629
<i>Bacillus subtilis</i>	1134
<i>Corynebacterium glutamicum</i>	
<i>Staphylococcus aureus</i>	
<i>Aspergillus niger</i>	281
<i>Salmonella typhi</i>	
<i>Salmonella typhi A</i>	
<i>Salmonella typhi B</i>	
<i>Pseudomonas aeruginosa</i>	847
<i>Proteus vulgaris</i>	426

strains are collected from MTCC, Gujarat University microbiology dept and M.G.Science college ahmedabad, they are deposited in the biotech research lab of MUIS, Ganpat University.

2. Please make table of antibacterial /antifungal activity of standard drugs against tested organisms  
Yet this work has to be done ; its under progress.
3. Please show the antibacterial and antifungal activity of the extract linearly increase in concentration of extract as compared with standard drugs  
This work also yet to be done. Its also under progress.