

## Antibacterial Activity and Phytochemical Analysis of *Euphorbia hirta* against Clinical Pathogens

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### ABSTRACT

*Euphorbia hirta* powdered plant material was extracted using two solvents distilled water and methanol. The water extract provide higher yield and also more antibacterial effectiveness than methanol extract used. The well diffusion method was used to determine the antibacterial activity against *Escherchia coli*, *Salmonella typhi*, *Bacillus subtilis*, *pseudomonas aeruginosa*, *Klebsiella pneumoniae*. *E.hirta* plant was investigated for some of their components and antimicrobial activity of their leave extract against bacteria. Phytochemical screening of the crude extracts revealed the presence of tannins, saponins, steroids, flavonoids, terpenoids, and alkaloids. In GC-MS analysis, four bioactive phytochemical compounds were identified in the methanolic extract of *Euphorbia hirta*, the components were identified by comparing their relation indices and mass spectra Fragmentation patterns with those stored on the MS-Computer library. This presence of these bioactive constituents has been linked to the antimicrobial activity of the plant material. Dry leaf extracts of *E. hirta* produced the highest zones of inhibition on *Salmonella typhi*. The herb *E. hirta* can be used as source of oral drugs to fight infections caused by susceptible bacteria.

**Keywords:** *E.hirta*, antibacterial activity, phytochemical analysis, GC-MS.

### 1. INTRODUCTION

Herbal medicine has been used in many parts of the world as a rich tradition for the treatment of a many infectious diseases (Brantner et al., 1994). In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care (Matu et al., 2003), because of better cultural acceptability, better compatibility with the human body and fewer side effects. Medicinal herbs have curative properties due to presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants (Kumar et al., 2010). *Euphorbia hirta* is annual prostrate weed which belongs to family Euphorbiaceae found mostly in India. *Euphorbia hirta* is commonly known as amman patcharisi by local people. It mainly grows in rainy season. It grows in open grasslands, road sides and pathways. The plant is also widely used in Angola against diarrhea and dysentery, especially amoebic dysentery. In Nigeria extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing (Igoli et al., 2005).

This study was undertaken to investigate the phytochemical properties and antibacterial activities of the plant against some economically important bacteria that cause a variety of intestinal and extra intestinal diseases.

## 2. MATERIALS AND METHODS

### Collection and preparation of plant material

The fresh plant *E. hirta* was collected around the surroundings of main campus of the St. Michael college of Engineering and Technology, Kalayarkoil, Tamilnadu state, India. The fresh plant was harvested, rinsed with tap water and air dried under shade for 4



**Fig 1:** *E. hirta* plant



**Fig 2:** Methanol Extract



**Fig 3:** Water Extract

Preliminary phytochemical analysis was done for the both methanol and water extract. Test for tannins, saponins, flavonoids, alkaloids and steroids were done.

GC-MS analysis of the extract was performed using a Perkin-Elmer GC Clarus 500 system. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass. Interpretation on mass spectrum GC-MS was conducted using the database of national Institute Standard and technology (NIST) having more than 62,000 patterns.

### Antibacterial Activity

Among five microorganisms one Gram positive *Bacillus subtilis* while four Gram negative bacteria were *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*. All the cultures maintained on nutrient agar slants at 40°C. The antibacterial assay activity was performed by agar well diffusion method. For agar well method, about 15-20 ml Muller Hinton agar was poured into sterilized petri plates. After solidification 0.2 ml of broth culture of test microorganism were inoculated in the media separately. 10 µl of test compound was introduced into the well. The plates were incubated overnight at 37°C. Microbial growth was determined

days and reduced to coarse powder using pestle and mortar and then grinded to fine powder. The powder was stored in an airtight bottle until needed for use. The preparation of extract was done by maceration method. Methanol and water used as solvent. Extraction from fresh and dry leaves of *E. hirta* was done with water and methanol.

by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented.

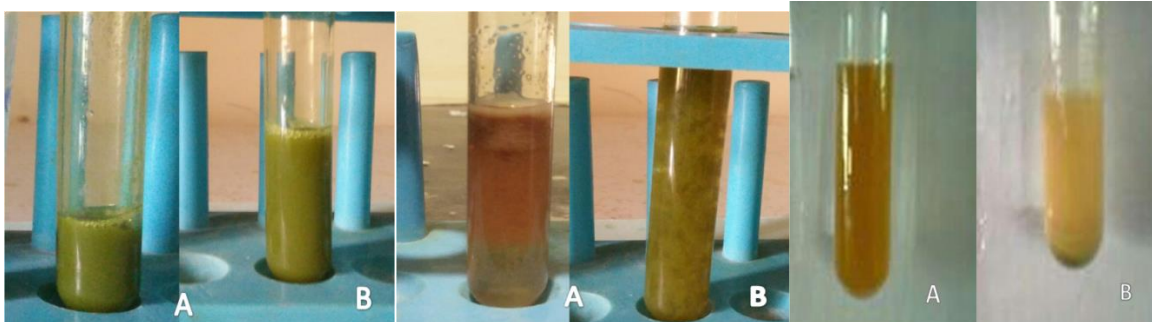
## 3. RESULTS AND DISCUSSION

### PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical screening of the crude extract of *Euphorbia hirta* showed the presence of saponins, tannins, alkaloids, flavonoid, and steroids. Table shows the phytochemical screening of aqueous, methanol, of *Euphorbia hirta*. Qualitative screening confirms the presence of five compounds support the use of plant in folklore medication.

PHYTO COMPOUNDS	WATER EXTRACT	METHANOL EXTRACT
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Steroids	+	+
Alkaloids	+	+

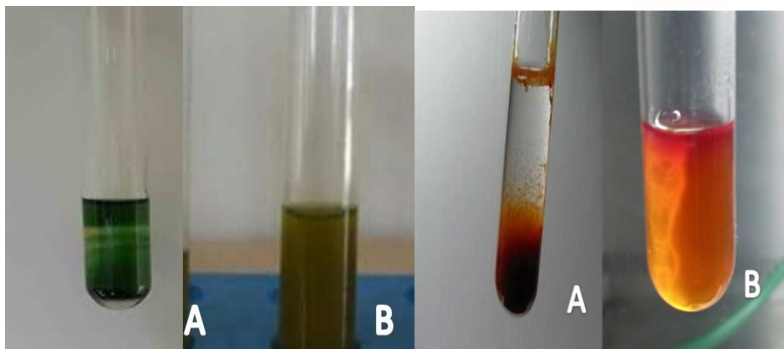
**Tab 1:** Phytochemical composition of water, methanol of *E. hirta*



**Fig 4: Test for Saponins**

**Fig 5: Test for Tannins**

**Fig 6: Test For Steroids**



A – Methanolextract, B- water extract

**Fig 7: Test for Flavanoids**

**Fig 8: Test For Alkaloids**

**GC-MS ANALYSIS**

The methanolic leaf extract of *Euphorbia hirta* was studied by GC-MS. There are 4 compounds identified using this technique. Among these, phytol, is a major compound because it is act as a skin care agent and anticancer agent .GC-MS chromatogram of the methanol extract of *Euphorbia hirta* showed four peaks which indicated the presence of four major phytochemical constituents. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula.

Sl. No	Name of compound	RT (min)	Peak Area(%)	% of equal	Molecular Weight	Molecular Formula
1	Azulene	6.021	2.98	93	128.17	C <sub>10</sub> H <sub>8</sub>
2	2-propenoic acid, 3-phenyl	9.038	6.20	97	148.0524	C <sub>9</sub> H <sub>8</sub> O <sub>2</sub>
3	Phytol	16.441	3.28	64	296.53	C <sub>20</sub> H <sub>40</sub> O
4	Bis(2-ethylhexyl)	20.091	87.54	91	390.56	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>

**Tab 2: Phytochemicals identified in the leaf methanol extract of *E.hirta***

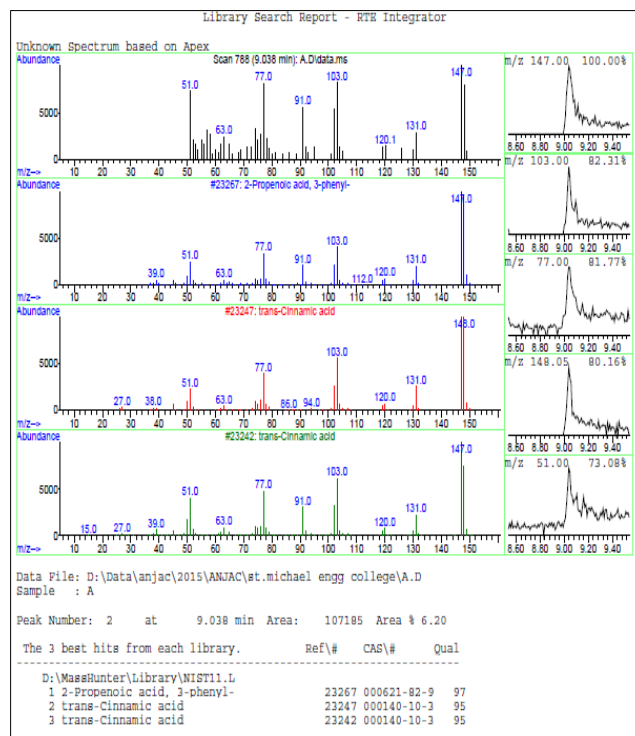
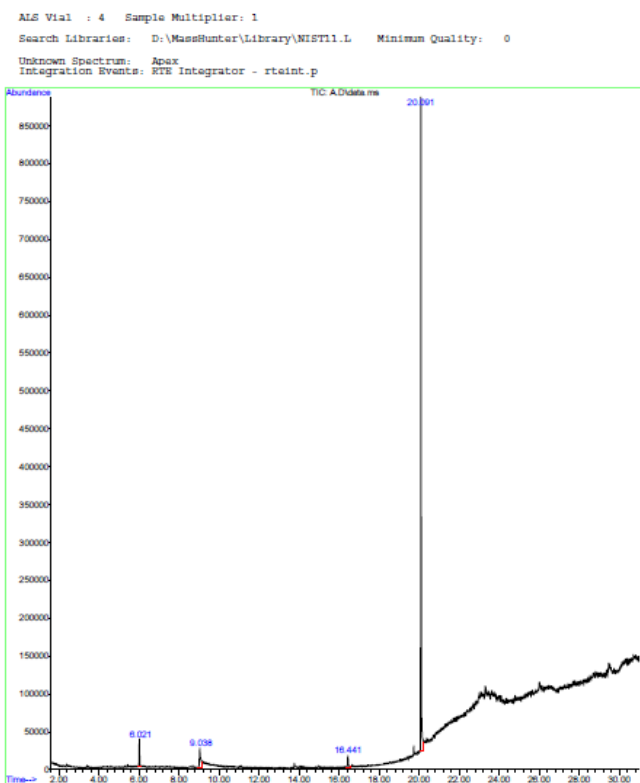


Fig 9: GC-MS chromatogram of methanolic extract of leave of *E.hirta*

Fig 11: Mass spectrum of 2-propenoic acid, 3-phenyl-

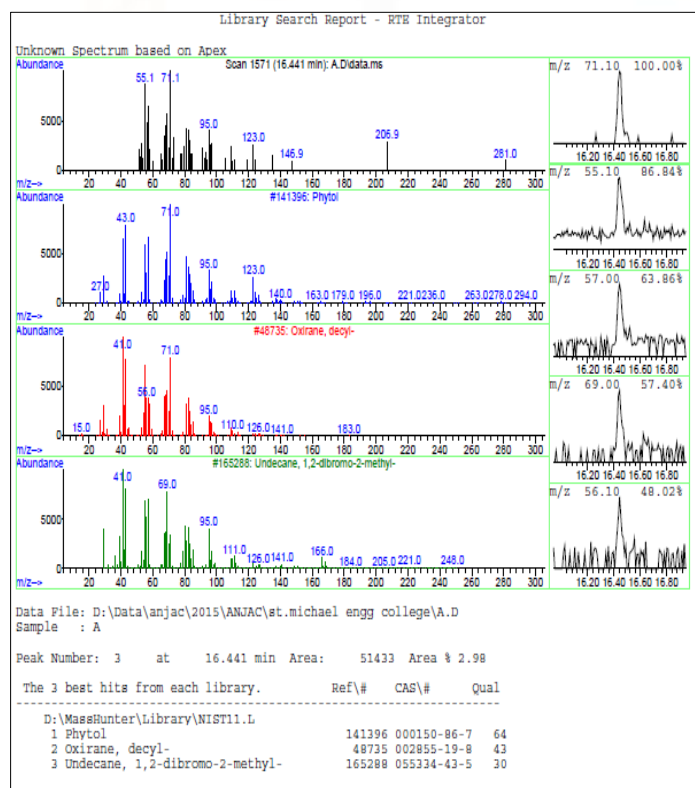
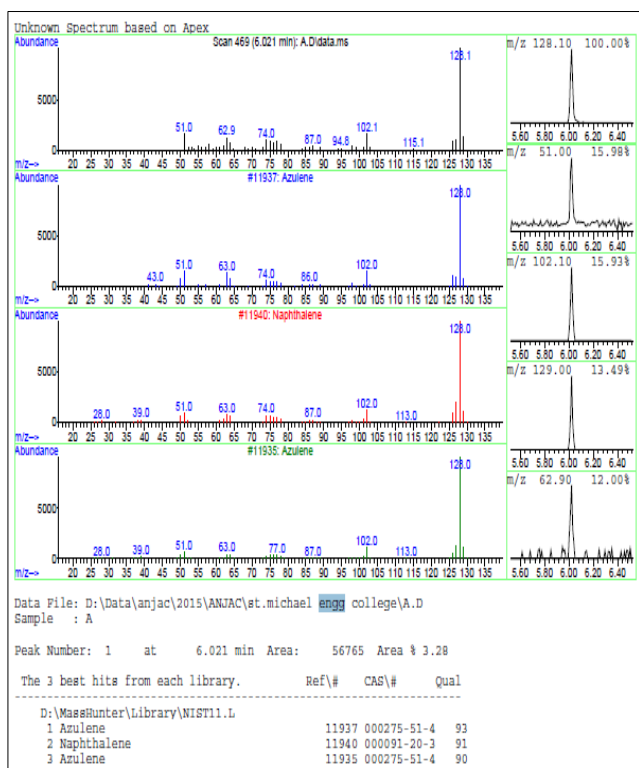


Fig 10: Mass spectrum of azulene

Fig 12: Mass spectrum of phytol

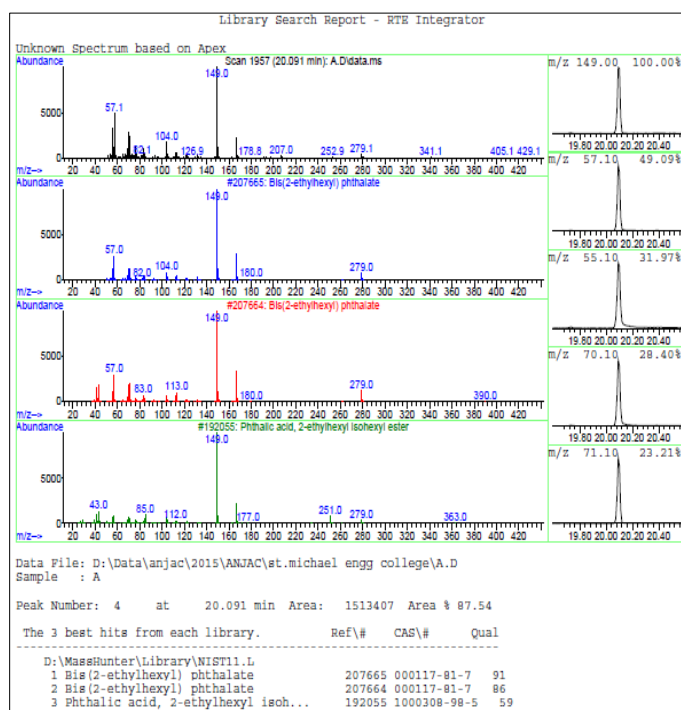


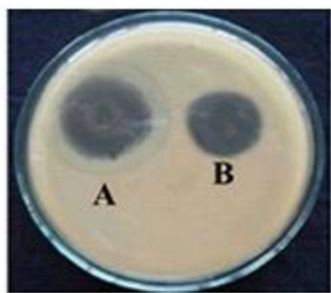
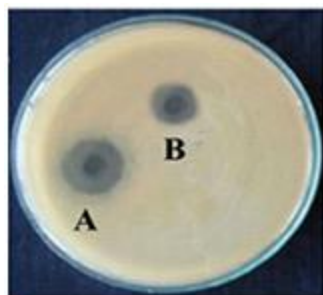
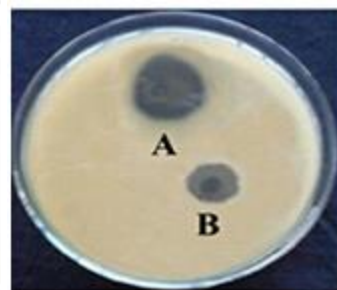
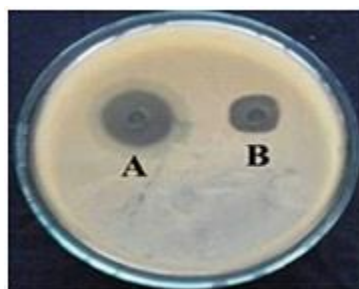
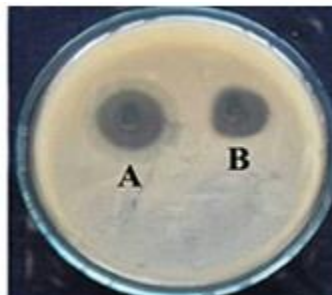
Fig 13: Mass spectrum of Bis (2-ethylhexyl)

### ANTIBACTERIAL ACTIVITY

The antimicrobial activities of *E. hirta* were assessed using the agar well diffusion method. All the pathogens used in this study were susceptible to both methanol and aqueous extracts of dry leaf of *Euphorbia hirta*.

The results indicated that at 20µl/ml, methanol extracts of the leaf produced the highest significant zone of inhibition (11.67mm) on *S.typhi* while the least zone of inhibition was observed on *P.aeruginosa* with an average value of 6.0mm. Similarly, the effect of the water extracts of the leaf on *K.pneumoniae* produced the least significant zone of inhibition. However, the highest effect was shown on *S.typhi* with mean values range is 10.12 mm.

ORGANISMS	CONCENTRATIONS (µl/ml)	ZONE OF INHIBITION DIAMETER (mm)	
		Methanol extract	Water extract
<i>E.coli</i>	20	8.4	6.6
<i>S.typhi</i>	20	11.67	10.12
<i>B.subtilis</i>	20	7.2	5.3
<i>K.pneumoniae</i>	20	7	5
<i>P.aeruginosa</i>	20	6	5.7

Fig 14: *E.coli*Fig 15: *S.typhi*Fig 16: *B.subtilis*Fig 17: *K.pneumoniae*Fig 18: *P.aeruginosa*

#### 4. CONCLUSION

The *Euphorbia hirta* plant showed antibacterial effect on common pathogen of human. *Euphorbia hirta* was found to contain some bioactive compounds with pronounced antibacterial activities, further Phytochemical and Pharmacological studies will be needed to isolate the active constituents and evaluate the antimicrobial activities against a wide range of microbial pathogens.

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