



REGULAR ARTICLE

## ANTIBACTERIAL EVALUATION OF SNAKE WEED (*EUPHORBIA HIRTA* L.)

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### SUMMARY

Ethanol, Methanol, Chloroform and Aqueous (water) extracts of leaf, stem, root and whole plant of *Euphorbia hirta* L. (Euphorbiaceae) were used to evaluate antibacterial activity. The agar-well diffusion assay was employed against several Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*) bacterial species. Aqueous and chloroform extracts of stem and root did not express any activity. Antibacterial activity was recorded in the order of ethanol, methanol, aqueous and chloroform extracts. Among these extracts ethanol and methanol extracts of leaf and whole plant were more effective and significant than aqueous and chloroform extracts in inhibiting the growth of the pathogenic bacteria under study, but were less potent when compared to that of tetracycline used as positive control. Phytochemical screening of the plant revealed the presence of tannins, flavonoids, alkaloids, glycosides and saponins. The study scientifically validates the use of plant in traditional system of medicine to treat various diseases.

**Keywords:** *Euphorbia hirta* (L.), antibacterial activity, micro-organisms.

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### 1. Introduction

In many parts of the world, there is a rich tradition in the use of herbal medicine for the treatment of many infectious diseases [1]. In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care [2]. Many currently used drugs are expensive or not readily available and a major set back to their continued usage is the development of resistance. This situation urgently forced scientists for searching new inexpensive drugs that will be able to act for longer periods before resistance set in. Because of the side effects and the resistance that pathogenic microorganisms build resistant against the common antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plants used in herbal medicine [3].

*Euphorbia hirta* (family: Euphorbiaceae) is an herb found in many parts of the world

and its common names, Australian asthma plant, Garden spurge and Snake weed. The stem and leaves produce white or milky juice when cut [4]. The leaves of *Euphorbia hirta* are found to contain flavonoids, polyphenols, tannins, sterols, alkaloids, glycosides and triterpenoides [5]. The plant has a reputation for increasing milk flow in women because of its milky latex and is used for other female complaints as well as diseases like bronchitis, asthma, eczema, dysentery. It is used as antidiarrheal, antispasmodic, anti-inflammatory, antifungal, anticancer, antimalarial, antiamebic, antibacterial and antihelminthic etc. The present investigation was carried out on different plant parts of *Euphorbia hirta* (L) (Root, stem, leaf and whole plant) to determine the antibacterial activity of their aqueous, alcoholic and chloroform extracts against five bacterial strains (both Gram-positive and Gram-negative).

## 2. Materials and Methods

### Collection of plant materials:

The different parts of *Euphorbia hirta* (L.) such as leaf, stem, root and whole plant were collected from the field grown plants in and around the Andhra University, Visakhapatnam, Andhra Pradesh and were used for the following experiments.

### Sample preparation and extraction procedure:

Freshly collected plant materials such as leaves, stem, root and whole plant of *Euphorbia hirta* (L.) were dried in shade in well - ventilated enclosures and ground into fine powder using a mechanical grinder. 50g of the fine powder of each plant part of *Euphorbia hirta* (L.) was extracted in Soxhlet apparatus separately [6]. Sequential extraction was done with methanol, ethanol, chloroform and distilled water for about 18 hours with each solvent. The extracts were evaporated to dryness under vacuum using a rotary evaporator. The extract was then stored below ambient temperature.

### Preparation of dilution of crude extract for antibacterial assay:

The methods of Akujobi *et al* [7] and Esimone *et al* [8] were adopted. The crude extracts were dissolved in 30% dimethyl sulphoxide (DMSO) and further diluted to obtain of each extract sample at 750 µg/ml concentration was used for the determination of antibacterial activities by agar - well diffusion method.

### Test micro organisms

The organisms *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus* were obtained from microbial type culture collection (MTCC), IMTECH, Chandigarh, India. They were re-isolated and the pure cultures subcultured on nutrient agar slants. They were stored at 4°C until required for the study.

### Evaluation of anti bacterial activity

The agar-well diffusion method as described by Esimone *et al* (1998) was adopted for the study. fifteen ml of molten nutrient agar was seeded with 1.0 ml of standardized broth cultures of the bacteria ( $1.0 \times 10^8$  CFU/ml) by introducing the broth cultures into sterile petridishes, incorporating the molten agar, rotating

slowly to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. Four wells (holes) were made in the plates (about 6.0 mm diameter) using sterile cork borer and equal volumes of the extracts were transferred into the wells using micropipette. Three petridishes containing a particular microorganism were used for each concentration of the extract. The plates were allowed to stand for one hour for pre-diffusion of the extract to occur and were incubated at 37°C for 24 hours. At the end of incubation the plates were collected and zones of inhibition that developed were measured. By average of the zone of inhibition was calculated. Antibiotic tetracycline at a concentration of 30µg/ml as positive control and 100% Dimethyl sulphoxide (DMSO) as negative control were used.

## 3. Results

The antibacterial activity of crude extracts of the different parts of *Euphorbia hirta* (L.) were assessed using the agar-well diffusion method by measuring the diameter of growth inhibition zones with aqueous (water), methanol, ethanol and chloroform extracts are depicted in Table- 1. In total of sixteen extracts belongs to different parts of *Euphorbia hirta* (L.) were tested against both Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*) bacteria in the present investigation. The aqueous and chloroform extracts of stem and root exhibited no bacterial activity, whereas the extracts of leaves and whole plant exhibited higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. Ethanol and methanol extracts of leaves and whole plant were found to be highly significant antibacterial activity against Gram-positive bacteria but moderate and significant activity against Gram-negative bacteria. Similarly the same extracts of the stem and root were found moderately significant antibacterial activities against Gram-positive bacteria. Chloroform extracts of leaf and whole plant exhibited moderately significant antibacterial activity against Gram-positive bacteria whereas stem and

root extracts exhibited lower and insignificant antibacterial activity against both Gram-positive and Gram-negative bacteria (Table 1).

Table 1. Antibacterial activity of different parts of *Euphorbia hirta* extracts.

Plant part	Plant extracts with different concentrations	Zone of inhibition(mm)				
		<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus vulgaris</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>
Leaves	Aqueous	5	4.5	6	9	8
	Methanol	12	9	10	16	14
	Ethanol	14	10	12	19	16
	Chloroform	4	2.5	3	8	6
Stem	Aqueous	--	--	--	--	--
	Methanol	8	6	7	13	11
	Ethanol	9	8	10	15	13
	Chloroform	--	--	--	--	--
Root	Aqueous	--	--	--	--	--
	Methanol	7	5	4	12	10
	Ethanol	8	6	7	14	12
	Chloroform	--	--	--	--	--
Whole plant	Aqueous	8	5	7	11	9
	Methanol	14	10	12	18	16
	Ethanol	15	12	14	24	21
	chloroform	6	3	7	10	7
Tetracycline (30µg/ml)		17	14	5	26	24

--: No activity

From the results of antibacterial screening of four solvents (ethanol, methanol, chloroform and water) of different parts of *Euphorbia hirta* (L.) used in this study ethanol and methanol extracts of whole plant and leaf exhibited the best antibacterial activity.

#### 4. Discussion

The presence of antibacterial substances in the higher plants is well established [9]. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in the study the plant extracts by ethanol and methanol provides more consistent

antibacterial activity compared to those extracted by water. The results of the antibacterial activity of different plant parts of *Euphorbia hirta* (L.) against the investigated bacterial strains are shown in the Table-1.

In the present study the results obtained indicated that the ethanol, methanol, chloroform and water extracts of different plant parts of *Euphorbia hirta* inhibited the growth of the tested microorganisms viz. *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris* (Gram-negative) and *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive). However, its effects are low when compared with standard antibacterial agent tetracycline was used in this study as positive control (Table 1). The present results revealed that the alcoholic extracts (ethanol and methanol) showed the maximum degree of inhibition of zone as compared with aqueous and chloroform extracts. It is quite possible that the stem and root extracts of water and chloroform were ineffective in the present study do not posses antibacterial

properties. This may be due to the active chemical constituents were not soluble in water and chloroform. Ethanolic and methanolic extracts of whole plant and leaf of *Euphorbia hirta* showed the maximum degree of antibacterial activity properties. This may be due to the presence of alkaloids, tannins, saponins and flavonoids which are plant secondary metabolites known to possess antibacterial properties. Similar observations were also reported in various plant extracts with different concentrations [7, 8, 10, 11, 12].

The higher resistance of Gram-negative bacteria to plant extracts as previously been documented and related to thick murein layer in their outer membrane, which prevents the entry of inhibitor substances [2, 13, 14, 15]. Similarly our results indicated that the antibacterial activities of the extracts of *Euphorbia hirta* were more pronounced on Gram-positive than on Gram-negative bacteria. Alternatively, the passage of the active compound through the Gram-negative cell wall may be inhibited that observed differences may result from doses used in the study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains.

The results of this study have shown the different plant parts of *Euphorbia hirta* have great potential as antimicrobial agents in the treatment of infectious diseases caused by resistance microorganisms. Further detailed study is necessary regarding individual screening of phyto constituents from the different plant parts of *Euphorbia hirta* to conclusively identify the antimicrobial compound. However, the present study indicates that the plant contains potential antimicrobial compounds which can be further developed as phytomedicine for prevention of infection.

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