



Antibacterial and phytochemical studies of *Euphorbia hirta* Linn. against respiratory tract pathogens

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Abstract

Euphorbia hirta was evaluated for antibacterial activity against human pathogenic bacterial strains *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* causing respiratory tract infections. Petroleum ether, acetone, methanol and water extracts of *E. hirta* were screened for antibacterial activity by cup-plate method at sample concentration of 200 mg/ml. The result of antibacterial activity revealed that methanolic extracts of the plant exhibited maximum activity as compared to petroleum ether, acetone and water. Minimum inhibitory concentration (MIC) of the methanolic extract of the plant was also calculated against the pathogens. *S. aureus* was the most susceptible bacteria. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, amino acids and steroids. The presence of these bioactive constituents has been linked to the antimicrobial activity of the plant.

Keywords: *Euphorbia hirta*, Antibacterial, Phytochemical, Respiratory tract infections

Introduction

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Iyengar, 1985; Chopra *et al.*, 1992; Harborne and Baxter, 1995). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens (Ahmad and Beg, 2001). *Euphorbia hirta* is one of such herbs belonging to the family Euphorbiaceae which is frequently seen occupying open waste spaces and grasslands, road sides and pathways. The plant is used in treatment of asthma and respiratory tract inflammations (Kokwaro, 1993). It is also used for coughs, chronic bronchitis and other pulmonary disorders in

Malagasy (Wong-Ting-Fook, 1980). The medicinal usefulness of the herb has been the subject of numerous chemical and microbiological studies. Some of the reported phytoconstituents of the herb included triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikimic acid, while some of the reported scientific uses include its use as an antispasmodic, antiasthmatic, expectorant, anticatarrhal and antisyphilitic (Burkill, 1994; Adedapo *et al.*, 2005; Falodun *et al.*, 2006). In the present study, we have selected whole plant of *Euphorbia hirta* to be screened against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae*.

The observed antibacterial activity was believed due to the presence of many biologically active constituents of plant which were identified in the extracts.

Materials and Method

Plant material

The plants were collected from Shivalik range of Himalayas in Haridwar. The plant was identified at Botanical Survey of India, Dehradun,

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Uttarakhand (India). The whole plant was washed by running tap water to remove the adhering unwanted material and cutted into small pieces, dried at room temperature and then powdered by using blender.

Extraction of plant material

The powdered plant material was loaded in soxhlet assembly and extracted in four different solvents *i.e.* petroleum ether, acetone, methanol and water for 72 hours by successive method. At the end of each extract, it was passed through Whatman filter paper No.40 and the filtrates were evaporated under reduced pressure.

Preparation of Plant Extracts

The extracts were prepared by immersing 200 gm of dried powdered material in 600 ml of the solvents *i.e.* petroleum ether, acetone, methanol and water by soxhlet apparatus. After removing the solvents, the crude extracts were stored in sterile bottles at 4°C until further use.

Culture media

Muller Hinton Agar Media No. 173 (Hi Media Pvt. Ltd., Mumbai, India) was used for screening of antimicrobial activity.

Microorganisms used

The bacterial strains *i.e.* *Staphylococcus aureus* (MTCC-1144), *Streptococcus pyogenes* (MTCC-422) and *Streptococcus pneumoniae* (MTCC-655) used in this study are related to respiratory tract infections.

Antibacterial activity

The cup-plate method was used to evaluate the antibacterial activity. This method depends upon the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing a solution of tested material (Perez *et al.*, 1990, Ahmad *et al.*, 1998). 0.1 ml of diluted inoculum (10^5 CFU/ml) of tested organism was mixed in Muller Agar Hinton Media, shaken and poured in sterilized petridishes. Wells of 8 mm diameter were punched into the agar medium and filled with 45 µl of plant extracts. All the solvents served as negative control. Each extract was

assayed in triplicate. The plates were incubated at 37°C for 24 hours. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured in millimeters (mm).

Determination of MIC

The minimum inhibitory concentration of the methanolic extract was determined by using the serial micro dilution method. The sterile Muller Hinton Broth bacterial suspension was added into microtiter plates. The extract in broth was used as negative control and bacterial suspensions were used as positive control. The turbidity of the wells in the microtiter plate was interpreted as visible growth of the microorganisms. The MIC values were taken as the lowest concentration of the extract in the wells of the microtiter plate that showed no turbidity after 18-24 hours of incubation at 37°C.

Phytochemical screening of the plant

Phytochemical screening was carried out of the plant material for the presence of bioactive components such as alkaloids, flavonoids, glycosides, amino acids, steroids, saponins, tannins and phenolics (Trease and Evans, 1996).

Results and Discussion

The zone of inhibition of the petroleum ether, acetone, methanol and water extracts (200 mg/ml) from the *E. hirta* plant against *S. aureus*, *S. pyogenes* and *S. pneumoniae* are shown in Table 1. The methanolic extract showed the maximum zone of inhibition against all the bacteria followed by water, acetone and petroleum ether. The methanolic extract is highly effective against all pathogens because more organic compounds were leached in this solvent. The zone of inhibition formed by petroleum ether extract is least effective. The methanolic extract was highly active against *S. aureus* (26 mm) followed by *S. pyogenes* (25 mm) and *S. pneumoniae* (22 mm) as compared to others. Water extract showed best activity against *S. aureus* and *S. pyogenes* (24 mm). Acetone is more effective in comparison to petroleum ether. Acetone showed maximum inhibition against *S. aureus* (23 mm) followed by *S. pyogenes* and *S. pneumoniae* (21 mm). Petroleum ether was found most effective against *S.*



pyogenes (20 mm) followed by *S. aureus* (18 mm) and *S. pneumoniae* (17 mm). The basis for their difference in susceptibility might be due to constituents present in the extract. The leaves of *E. hirta* have considerable antibacterial activity against *Escherichia coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Ogbolie *et al.*, 2007).

Table. 1. Antibacterial activity of *Euphorbia hirta* extracts (mm) in different solvents

S.No.	Plant Extract	<i>S.aureus</i>	<i>S.pyogenes</i>	<i>S.pneumoniae</i>
1.	Petroleum ether	18	20	17
2.	Acetone	23	21	21
3.	Methanol	26	25	22
4.	Water	24	24	21

Values represent average of three replicates

The methanolic and aqueous extracts of *E. hirta* showed antimicrobial activity against *E. coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi* and *Proteus mirabilis*. The growth of all the bacteria was inhibited though to varying degree, thus justifying the use of the herb in traditional medicine (El- Mahmood, 2009). The ethanolic extract of *E. hirta* (root) had strong inhibitory effect against *Propionibacterium acnes* (Kumar *et al.*, 2007).

The phytochemical screening of the plant extracts showed the presence of alkaloids, flavonoids, glycosides, amino acids, steroids, saponins, tannins and phenolics (Table-2). Alkaloid was present in acetone and methanolic extracts of the plant whereas absent in petroleum ether and water extracts. Methanolic extract of the plant possess the presence of maximum constituents. Phytochemical screening of the crude extracts of *E. hirta* revealed the presence of tannins, saponins, phenolics, cardiac glycosides, anthraquinones, flavonoids and alkaloids. These compounds have potentially significant application against human pathogens, including those that cause enteric infections (El-Mahmood *et al.*, 2008).

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in extraction procedure. Traditional healers use primarily water as the solvents (Ahmad *et al.*, 1998) but in our studies we found that plant extracts in organic solvents (methanol) provided more consistent antimicrobial activity as compared to those extracted in water.

Table. 2. The phytochemical screening of *Euphorbia hirta*

S.No.	Plant	Alkaloids	Flavonoids	Tannins/ Phenols	Saponins	Glycosides	Amino acids	Steroids
1.	Petroleum ether	-	-	-	-	-	-	+
2.	Acetone	+	-	+	-	-	-	+
3.	Methanol	+	+	+	+	+	+	+
4.	Water	-	+	+	+	-	+	-

+ = present - = Absent

These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity by their ability to dissolve or diffuse in the media used in the assay. The observed antibacterial effects on the isolates are believed to be due to the presence of flavonoids, alkaloids and tannins which have been shown to possess antibacterial properties (Ogbolie *et al.*, 2007, Cowan, 1999, Draughan, 2004). Newze *et al.* (2004) have also attributed observed antimicrobial effects of plant extract to the presence of these secondary metabolites. In conclusion, the extract of *E. hirta* plant has high potential as antibacterial agents. This finding has validated the use of these medicinal plants for the treatment of microbial infections. It seems important to recommend that further studies using isolated constituents instead of whole extracts must be done in this field.

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