

# ANTIMICROBIAL ACTIVITY OF EUPHORBIA HIRTA L.

#### Bismi Sonia Nazeer Guest Lecturer, Department of Botany, Bishop Moore College, Mavelikkara

The antibacterial and antifungal effect of Euphorbia hirta was evaluated against a panel of bacteria (Escherichia coli, 5 Psueudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus) and fungi (Aspergillus flavus, Fusarium RA semitectum and Trichophyton sp.). E. hirta methnol extract was more sensitive against both bacteria and fungi tested. F. semitectum (27.12mm) showed higher sensitivity followed by E. coli (22mm), F. semitectum (21.80mm). P. aeriginosa, Trichophyton sp. (acetone extract) and S. aureus (fresh latex) are resistant. The plant can be used to discover new bioactive natural compounds that may serve as leads in the development of new pharmaceuticals with less side effects and resistance risks.

## **KEYWORDS**

EUPHORBIA HIRTA, ANTIBACTERIAL, ANTIFUNGAL, DRUG RESISTANCE

### INTRODUCTION

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The use of plants to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics is as old as the human species. In the developing countries about 70-95% people still rely on natural medicines for healing (Ahmad and Wajid, 2013). The unprecedented emergence of microbial resistance to antimicrobials and side effects associated synthetic antimicrobial agents, has resulted in increased interest in plant derived extracts or compounds recently (Blair et al., 2015; Sharififar et al., 2016). Thus, there is an urgent need to search for new infection-fighting strategies to combat microbial infections in the current era of emerging infectious diseases.

Euphorbia hirta L. (family Euphorbiaceae) is a pan tropical annual weed found especially on roadsides and wastelands. The plant is commonly known as Amman pachirisi in Tamil and also called as Tawa-tawa, snake weed and asthma weed. The plant is widely used in traditional medicine to cure various diseases, especially gastrointestinal disorders, afflictions of the skin and mucous membranes and respiratory system disorders (Huang et al., 2012). E. hirta is mainly rich in gallic acid, quercetin and a phenoilc substance C<sub>28</sub>H<sub>18</sub>O<sub>15</sub>. Several studies revealed that *E. hirta* possess anti-anaphylactic, antioxidant, anticancer, antifeedant, antiplatelet aggregation and anti-inflammatory, aflatoxin inhibition, antifertility, antiplasmodial, antiamoebic, larvicidal, and insect repellent activities (Lanhers et al., 1991; Sandeep et al., 2009; Widharna et al., 2010; Sandeep and Chandrakant, 2011). The present study is an attempt to evaluate the antimicrobial activities of the fresh latex, methanol and acetone extract of Euphorbia hirta against a panel of bacteria (Escherichia coli, Psueudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus) and fungi (Aspergillus flavus, Fusarium semitectum and Trichophyton sp.)

# MATERIALS AND METHODS

#### Extract preparation

The plant *E. hirta* grows upto a height of 40cm tall and it can also be seen lying down. The stem is slender, reddish in colour. Leaves simple, arranged oppositely, distichous, leaf blades are lanceolate, unequal base, cuneate one side, round otherside, acute apex, finally toothed margins, dark green above, pale beneath, purple bloth in middle, measures about 1-2.5 cm long. Flowers unisexual, male flowers are sessile, linear bracteoles, fringed, single stamen, with absent perianth. Female flowers are short pedical, rimmed perianth, superior ovary, three-celled, three styles, minute, covered with short hairs, two-fid apex. Inflorescence - cyathium at terminal or auxillary. Several cyathia densely clustered into a cyme.

The plant was collected from different regions in Kottyam District and taxonomically identified by using standard taxonomic keys

and expert consultations. The collected plants were dried under shade, crushed and subject to soxhlet extraction with methanol and acetone. The extract was filtered and concentrated. Fresh latex was collected from the stems early in the morning, rinsed with distilled water and minced slightly and juice was obtained by the squeezing the mass. Collected juice was filtered and subjected immediately for antimicrobial activity.

#### Antimicrobial susceptibility testing

Pure cultures of bacteria (Escherichia coli, Psueudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus) and fungi (Aspergillus flavus, Fusarium semitectum and Trichophyton sp.) were used for the study. Bacterial strains were maintained on nutrient agar slants at 4°C. A loopful of each bacterial strain was added to a 50 ml sterile nutrient broth in a 100 ml conical flask. The flasks were then incubated for 24 h to activate the test strain. Pure cultures of fungi were maintained on Sabouraud Dextrose Agar (SDA) slants at 4°C. It was subcultured on to SDA plates and incubated at room temperature for 5-8 days. The developed spores were harvested and spore suspension was used for the antimicrobial assays.

#### Agar diffusion method

The antimicrobial activity of the plant extracts (methanol and acetone) and fresh latex was assessed by the agar diffusion method (Balouiri et al., 2016). The agar plate surface is inoculated by spreading of the bacterial inoculum uniformly over the entire agar surface. For fungi, SDA was inoculated with fungal spore suspension at bearable temperature and transferred to sterile petri plates. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer and a volume (50  $\mu$ L) of the extract was introduced into the well. Then, the bacterial plates are incubated overnight. Similarly, the fungal plates were incubated at room temperature for 5 - 10 days. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. The antimicrobial activity was determined by measuring the zone of inhibition and expressed as millimeter (mm) (Mahmoudabadi and Nasery, 2009). Five sets of plates are used for the antimicrobial studies along with control plates.

#### RESULTS

The antibacterial and antifungal effect of *E. hirta* was evaluated against a panel of bacteria (Escherichia coli, Psueudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus) and fungi (Aspergillus flavus, Fusarium semitectum and Trichophyton sp.). The sensitivity pattern was given in Table 1. E. hirta methnol extract was more sensitive against both bacteria and fungi tested. F. semitectum (methanol extract) (27.12mm) showed higher sensitivity followed by E. coli (methanol extract) (22mm), F. semitectum (acetone extract) (21.80mm). P. aeriginosa,

Bacteria tested			
	Methanol extract	Acetone extract	Fresh latex
	Zone size in mm		
Escherichia coli	22±1.04	16	20.23±1.50
Psueudomonas aeruginosa	20.17±1		19.50±2.89
Klebsiella pneumoniae	19	17.01±2.30	15
Staphylococcus aureus	17.81±3.61	16.80±1.90	
Fungi tested			
Aspergillus flavus	20.75±2.95	17.20±1.84	20
Fusarium semitectum	27.12±1.40	21.80±3.71	19.57±1.27
Trichophyton sp.	19.69±1.53		22

Table 1: Antimicrobial effect of E. hirta extracts



Photo 1: Effect of Methanol extract on F. semitectum



Photo 2: Effect of Methanol extract on K. pneumoniae

#### DISCUSSION

Plants and plant based products are used in the treatment and prevention of illness from time immemorial. The secondary metabolites plants are the cornerstone of many treatment modalities like Homeopathy, Allopathy, Unani - Ayurvedic medicine (Altundağ and Öztürk, 2011). The genus Euphorbia is a member of family Euphorbiaceae, comprising about 2000 species occupying a multitude of habitats. The presence of latex is a unique characteristic of the family. The plant is routinely used by traditional healers to combat various ailments like amebiasis, diarrhea, peptic ulcers, heartburn, vomiting, afflictions of the skin and mucous membranes and respiratory disorders, coughs and colds) (Patil *et al.*, 2009; Huang *et al.*, 2012).

The present study is a attempt to evaluate the antibacterial and antifungal effect of E. hirta was evaluated against a panel of bacteria (*Escherichia coli, Psueudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus*) and fungi (*Aspergillus flavus, Fusarium semitectum* and *Trichophyton sp.*). The obtained results are at par with the available reports. Rajasekar et al. (2015) evaluated the efficacy of *Euphorbia hirta* against Mycobacterium tuberculosis and other non mycobacterial pathogens (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Vibrio cholerae* and *Enterococcus faecalis*) with promising results.

In another study, Hussain et al. (2014) reported the antimicrobial

activity of fresh latex of *Euphorbia hirta* against *Bacillus pumilus* (24.98),*Staphylococcus aureus* (25.38), Streptococcus *pneumonia* (23.72), *Escherichia coli* (27.93), *Citrobacter freundii* (23.54) and *Klebsiella pneumoniae* (21.93) which are also in agreement with the present findings.

The antifungal activity of *E. hirta* was well established and the present findings are also in accordance with previous reports. The antifungal activity of ethyl acetate extract of the inflorescence of *E. hirta* was studied with commendable activity targeting the cell membrane which could result in leakage of cellular proteins (Gayathri and Ramesh, 2013).

The search for novel natural products from medicinal plants against multidrug resistant microbial strains is promising and urgent need of the hour, as drug resistance issues are emerging alarmingly. The plant is rich in flavonoids, terpenoids, phenols, essential oil, and other compounds like gallic acid, quercetin and a phenoilc substance  $C_{28}H_{18}O_{15}$  which pave the way for the antimicrobial effects.

#### CONCLUSION

*E. hirta* is a popular herb among traditional practitioners for the treatment of various ailments with versatile usages among different regions. The study can be used as a stepping stone to combat emerging infectious diseases and also emerging drug resistance paradigms and more works need to be done with the view of their use for *in-vivo* studies.

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