

Antimicrobial Screening of Euphorbia hirta L. and Pedalium murex L. - A Comparative Study

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ABSTRACT

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Herbal medicine is still mainstay of about 75-80 % of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatiability with the human body and fewer side effects. *Euphorbia hirta* (amman pacharisi) is a small perennial herb which belongs to the family of Euphorbiaceae and it is used to treat bronchitic asthma, ameoboidal dysentry and laryngeal spasm. *Pedalium murex* (yanai nerunjil) is a much-branched annual herb. This belongs to the family of Pedaliaceae and is used to treat gonohorrea, urinary track infections and spermatohorrea. The present study is to compare the antibacterial and antifungal activity of aqueous and organic solvent leaf extracts of *Euphorbia hirta* and *Pedalium murex*The antimicrobial assay is done by disc diffusion method []against bacterial species (*Pseudomonas putida*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Aeromonas liquefaciens* and *Icaligenes* spp.) and fungal species (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus erythrocephalus* and *Fusarium* spp.).



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Introduction

Euphorbia hirta is a pantropical weed, possibly native to India. It is a hairy herb that grows in open grasslands, roadsides and pathways. It is widely used as a medicinal herb in most places it grows. In the test tube, Euphorbia hirta has been shown to kill various types of pathogenic bacteria, Plasmodium (potently) Euphorbia hirta has been claimed to have a curative effects on dengue patients, based on personal testimonies, particularly in the Philippines. Despite its widespread use, there is no evidence to support this claim (Srinivas et al, 2011, Yoganarasimhan S.N. 2000) **Pedalium** is a genus of plant in the Pedaliacae family comprising one species, **Pedalium murex**. It is distributed in India, Sri Lanka and Tropical Africa. **Pedalium Murex** is a fruit-bearing herb that has its history intertwined with Tribulus terrestris due to the visual similarities of the two fruits and their traditional interchanging in **Goshukra** formulas. Pedalium fruits are much less studied than Tribulus fruits currently. Preliminary evidence suggests that Pedalium could increase testosterone and act as an aphrodisiac, although there are no human studies currently. (Sankara Subramanian 1972, Yogendra et al 1983) The exact bioactives in Pedalium are equally unknown, and all that can be said is that many of the bioactivities of Pedalium are somewhat similar to Tribulus. (Katewa ss *et al* 2005. Balakrishnan V *et al* 2009. Dipali and Das 1995. Singh *et al* 2010.)

MATERIALS AND METHODS

Collection of Plant Materials:

The fresh and disease free plant leaves were collected from fallow land and wayside thorny shrubs, of Thanjavur, Tamilnadu, India and taken to laboratory. The herbarium specimens of these plants were deposited in medicinal plants herbarium, Trichy.

Microorganism:

The human pathogenic bacterial species [Pseudomonas putida, Pseudomonas aeruginosa, Klebsiella pneumoniae, Alcaligenes spp. and Aeromonas liquefaciens] and fungal species [Aspergillus niger, Aspergillus flavus, Aspergillus erythrochephalus, Aspergillus fumigatus and Fusarium spp.] were collected from the Microbial Culture Collection Unit (MCCU), Herbarium Reseach Unit, trichy, Tamil Nadu.

Preparation of Plant Extracts

The leaves of both plants [Euphorbia hirta and Pedalium murex] were surface sterilized with running tap water (3 times) and soaked in 0.1% mercuric chloride solution. Finally, the leaves were washed with distilled water and shade dried.

About 1g of leaves were grounded in mortar and pestle with 10ml of aqueous and organic solvents [acetone, chloroform, benzene, butanol, ethanol, dimethylformamide and diethyl ether] and centrifuged at 10,000rpm for 15 minutes. The supernatant was obtained and used for in vitro antimicrobial screening.

Antimicrobial Assay

The antimicrobial activity of the plant extracts were tested using disc diffusion method (Bauer et at 1966.Thornsberry et al 1988.)

Testing of Antibacterial activity

The sterilized Nutrient Agar medium (10ml) was poured into each sterile petriplate and allowed to solidify. The sterile cotton swab was dipped into the young bacterial cultures (20 hours) and swabbed over the entire agar surface on each petriplate. The sterile discs (5mm diameter) were loaded with 10μ l of each plant extract and kept undisturbed for the evaporation of solvents. Then, the discs were placed on the center of the petriplates and incubated at 37°C for 24hours. The antibacterial activity was recorded by measuring the diameter of inhibition zone around each disc (in mm).

Testing of antifungal activity

The above same procedure was repeated for testing antifungal activity. Instead of Nutrient agar medium, Potato Dextrose agar medium was poured into the sterile petriplates and then swabbed with young fungal cultures. Then, the discs (5mm diameter) with plant extracts were placed on the center for 3 days. The diameter of the inhibition zone was measured and its show antifungal activity. Acetone and butanol extracts of both the plants showed moderate activity. The other organic solvent extracts of both the plants showed moderate to least activity. Diethyl ether extracts of both plants was inactive against tested bacterial strains. The aqueous extract showed moderate activity in *Pedalium murex*, while *Euphorbia hirta* had least activity against single organism (*Pseudomonas aeruginosa*) and others are found to be inactive

RESULTS AND DISCUSSION

Efficacy of Euphorbia hirta and Pedalium murex against pathogenic bacterial strains: The results of the in vitro assays of antibacterial activity of selected medicinal plant extracts against bacterial strains (Table-1&2) Among the organism tested, Pseudomonas putida was strongly inhibited by both the plants. The other organisms reflect moderate activity. Among the different solvent used, ethanolic extract (Euphorbia hirta) and dimethyl formamide extract (Pedalium murex) contributed excellent activity against all the tested bacterial pathogens. Acetone and butanol extracts of both the plants showed moderate activity. The other organic solvent extracts of both the plants showed moderate to least activity. Diethyl ether extracts of both plants was inactive against tested bacterial strains. The aqueous extract showed



moderate activity in *Pedalium murex*, while *Euphorbia hirta* had least activity against single organism (*Pseudomonas aeruginosa*) and others are found to be inactive. Efficacy of *Euphorbia hirta* and *Pedalium murex* against pathogenic fungal strains: The results of the in vitro assay of antifungal activity against the tested fungal pathogens (Table-3&4). Among the five organisms tested, *Fusarium spp.*, was inhibited by both the plant extracts. The other organisms showed moderate to least activity. *Aspergillus niger* cannot be inhibited by both the plant extracts. Among the solvents used, butanol and ethanol extracts (*Pedalium murex*) showed some activity, while dimethyl formamide and benzene extracts (*Euphorbia hirta*) contributed excellent activity against all tested fungal pathogens. The other solvent extracts of both the plants showed moderate to least activity. The chloroform, diethyl ether and aqueous extracts of both the plants had no activity against all the tested fungal strains.

SUMMARY AND CONCLUSION

The Euphorbia hirta extracts had better antifungal activity, but moderate antibacterial activity while, the Pedalium murex extracts expressed significant antibacterial activity but least antifungal activity. From our screening experiments, the results showed that the most active constituents present in these plants can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation

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Table: 1- Antibacterial Activity of Leaf Extracts of *Euphorbia hirta*

Solvents Used	Inhibiotion of growth (Diameter in mm)					
	Pseudomonas Putida	Pseudomonas aeruginosa	Klebsiella pneumoniae	Aeromonas liquefaciens	Alcaligenes spp.	
Acetone	10	11	11	13	11	
Ethanol	14	12	12	14	10	
Chloroform	11	9	8	12	11	
Dimethyl formamide	10	11	9	11	9	
Water	0	10	0	0	0	
Diethyl ether	0	8	0	0	0	
Butanol	11	11	11	11	12	
Benzene	11	10	8	11	11	
Streptomycin (15mcg/disc)	23	11		22	24	



Table: 2 - Antibacterial Activity of Leaf Extracts of *Pedalium murex*

Solvents Used	Inhibition of Growth (Diameter in mm)					
	Pseudomonas Putida	Pseudomonas aeruginosa	Klebsiella pneumoniae	Aeromonas liquefaciens	Alcaligenes spp.	
Acetone	18	20	10	10	10	
Ethanol	16	15	12	12	12	
Chloroform	9	10	8	10	8	
Dimethyl formamide	18	11	18	11	17	
Water	9	0	9	11	9	
Diethyl ether	0	0	10	0	0	
Butanol	14	13	12	16	14	
Benzene	7	10	0	8	8	
Streptomycin (15mcg/disc)	23	11		22	24	



Table: 3 - Antifungal Activity of Leaf Extracts of Euphorbia hirta

A a a a a a sille e a ci a c			Inhibition of Growth (Diameter in mm)					
Aspergillus niger	Aspergillus flavus	Aspergillus fumigatus	Aspergillus erythrocephalus	Fusarium spp.				
0	7	7	7	16				
0	8	13	10	14				
0	0	0	0	12				
10	10	9	13	15				
0	0	0	0	0				
0	0	0	0	0				
0	12	10	7	15				
8	8	9	16	11				
	0 0 10 0 0	0 8 0 0 10 10 0 0 0 0 0 12 8 8	0 8 13 0 0 0 10 10 9 0 0 0 0 0 0 0 12 10 8 8 9	0 8 13 10 0 0 0 0 10 10 9 13 0 0 0 0 0 0 0 0 0 12 10 7 8 8 9 16				



Table: 4 - Antifungal Activity of Leaf Extracts of *Pedalium murex*

Solvents Used	Inhibition of Growth (Diameter in mm)				
	Aspergillus niger	Aspergillus flavus	Aspergillus fumigatus	Aspergillus erythrocephalus	Fusarium spp.
Acetone	0	0	0	0	0
Ethanol	0	12	13	8	8
Chloroform	0	0	0	0	0
Dimethyl formamide	0	0	8	0	11
Water	0	0	0	0	0
Diethyl ether	0	0	0	0	0
Butanol	8	10	0	12	13
Benzene	0	0	0	10	10