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RESEARCH ARTICLE

NATURAL PRODUCT

EFFICACY OF *EUPHORBIA TIRUCALLI* (L.) TOWARDS MICROBICIDAL ACTIVITY AGAINST HUMAN PATHOGENS

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ABSTRACT

The knowledge of plants as therapeutic agents is as old as disease, which in turn is as ancient as man himself. Since times immemorial plants have been in use to cure the human ailments. In traditional medicine, many plants have provided valuable clues for being used as potentially antiparasitic, antimalarial, leishmanicidal, antitumorous, fungicidal and antibacterial compounds. Still number of plants has yet to be screened for their potential as medicinal plants. In accordance with this information, antimicrobial activity of *Euphorbia tirucalli* (L.) was tested, which is commonly considered as medicinal plant. Extracts of the stem portions of the plant made in acetone, chloroform, hexane, methanol and petroleum ether were tested for antimicrobial activity against *Bacillus megaterium, B. subtilis*, *Escherichia coli, Enterobacter faecalis*, *Proteus vulgaris*, *Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus niger, A. fumigatus* and *Candida albicans.* The acetone extracts were effective against the test organisms.

KEY WORDS

Euphorbia tirucalli (L.), solvent extracts, antimicrobial activity.

INTRODUCTION

The use of plants as medicines is as old as human civilization itself. Many of the existing medicinal systems such as Ayurveda, Unani, Homeopathy, Naturopathy, Siddha and other alternative medicinal systems have been utilizing plants as effective medicines to cure many harmful diseases. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world[1]. *Euphorbia tirucalli L.* belongs to the family Euphorbiaceae. A large unarmed shrub or a small tree up to 5 m tall with erect branches; bark rough, cracked, greenish brown, exuding a milky sap when cut, branch lets slender, smooth, cylindrical, polished, whorled and modified into phylloclade[2]. The plant is used to treat gonorrhea, whooping cough, asthma, leprosy, enlargement of spleen, jaundice, tumors and bladder stones. Stem latex is used to treat warts, tooth ache, cough, asthma, ear ache, leprosy, abdominal pain, tumors, rheumatism, skin diseases and intestinal worms. Root is used for colic pains[3].

The principle aim of the work was to study the antimicrobial activity of *Euphorbia tirucalli* (L.) stem extracts in different solvents such as acetone, chloroform, hexane, methanol and petroleum ether against seven species of bacteria and three species of fungi. In the experimental study different fractions of solvent extracts of stem of the plant have been investigated.

MATERIALS AND METHODS

(i) PLANT MATERIAL

The *Euphorbia tirucalli* (L.) was collected from Gorantla, Guntur district of Andhra Pradesh, India in November, 2005. The collected material was authenticated by Dr.T.Pullaiah, Professor, Department of Botany and Biotechnology, Sri Krishna Devaraya University, Anathapur. A voucher specimen has been deposited at Acharya Nagarjuna University (ANU, DOB/BH/ET-001).

(ii) EXTRACT PREPARATION

Fresh plant material was washed thoroughly under running tap water, shade dried and used for extraction. The dried stems were homogenized to a fine powder and stored in airtight bottles. 25 g of stem powder was extracted with 150 ml of solvent (acetone, chloroform, hexane, methanol and petroleum ether) for 24 h by using soxhlet apparatus. The extract was dried in a flash evaporator for 30 min and the left over powder was considered 100%. Different concentrations such as 100, 250, 500, 750 and 1000 µg/ml were prepared by redisolving the extract powder in the same solvent which was used in the extraction.

(iii) TEST ORGANISMS

Tests were performed on seven species of selected bacteria such as *Bacillus megaterium* (ATCC 23564), *B. subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Enterobacter faecalis* (ATCC 35550), *Proteus vulgaris* (ATCC 638), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and three fungal species - *Asperigillus niger* (NCIM 596), *A. fumigatus* (NCIM 291) and *Candida albicans* (NCIM 670).The characteristics and diseases caused by these microorganisms were listed

under -Table No.1. All the test bacterial species were maintained on nutrient agar medium. 36 h old bacterial cultures was inoculated into nutrient broth and incubated on a rotary shaker at 35 ± 2 °C at 100 rpm. After 36 h of incubation, the bacterial suspension was centrifuged at 10000 rpm for 15 min. The pellet was resuspended in sterile distilled water and the concentration was adjusted to 1×10^8 cfu/ml using UV Visible Spectrophotometer. By reading the OD of the solution to 0.45Å (610nm) it was used for further studies. Fungal colonies were harvested from 9 -10days old cultures, which were maintained on potato dextrose agar. The spores were suspended in sterile distilled water and the spore suspensions were adjusted to 1×10^8 spores/ml (NCCLS) [4].

Table No: 1
List of the selected test organisms (bacteria and fungi)

S. No	Name of the Microorganisms	Characteristic feature	Diseases caused by the organisms
1	*Bacillus megaterium* (ATCC 23564)	Gram + ve	Intestinal disturbances.
2	*Bacillus subtilis* (ATCC 6633)	Gram + ve	Food poisoning, Oppurtunistic Pathogen.
3	*Escherichia coli* (ATCC 25922)	Gram - ve	Gastroenteritis, Urinary tract disease.
4	*Enterobacter faecalis* (ATCC 35550)	Gram - ve	Oppurtunistic human pathogen.
5	*Proteus vulgaris* (ATCC 6380)	Gram - ve	Urinary tract infections.
6	*Pseudomonas aeruginosa* (ATCC 27853)	Gram - ve	Wounds and urinary tract infections.
7	*Staphylococcus aureus* (ATCC 25923)	Gram + ve	Chronic osteomyletis, Meningitis, endocarditis.
8	*Aspergillus niger* (NCIM 596)	Dichotomously branching, filamentous	Allergy, Asthma
9	*Aspergillus fumigatus* (NCIM 291)	Monomorphic filamentous fungi	Pulmonary haemorrhage, pneumonia.
10	*Candida albicans* (NCIM 670)	Dimorphic fungi	Oral thrush, Gastritis, Cutaneous infection.

(iv) ANTIMICROBIAL ASSAY

Different concentrations of solvent extracts were tested for antimicrobial activity by using antibiotic sensitivity test. Microbial suspension was evenly mixed with sterile agar medium and poured into the sterile petri plates. After allowing the media to solidify at room temperature, wells of 6mm diameter were bored in the agar with sterile cork borer. Each concentration was checked for antimicrobial activity by introducing equal amounts of the sample (40µl) into wells. The method was repeated in five plates. Plates were allowed to stand at room temperature for 1 h and incubated at 37 °C for 24 to 48 h. The zone of growth inhibition around the wells was measured and area of inhibition zone was calculated. Simultaneously the activity of seven standard antibiotics such as Streptomycin (10µg/ml), Gentamycin (10µg/ml), Cholorompheniсol (30µg/ml), Vancomycin (10µg/ml), Rifimapicin (5µg/ml), Kanamycin (10µg/ml) and Nystatin (10µg/ml) were also tested against seven species of bacteria and three species of fungi under studying in similar conditions so as to compare the degree of inhibition by the solvent extracts. Agar wells fed with corresponding solvents served as control minimum inhibitory concentration which was determined as the lowest concentration on solvent extracts inhibiting the growth of organisms and was determined based on the readings.

RESULTS AND DISCUSSION

As Table No.2 indicates the acetone extracts of the stem of *E. tirucalli* were inhibitory to all the test microorganisms. *E. coli* was found to be highly sensitive to the acetone extracts of *E. tirucalli.* The MIC was 500 µg for *C. albicans* and 750 µg for *A. niger* and *A. fumigatus.* The chloroform extracts of the stem of *E. tirucalli* are active against *B. subtilis, E. coli, P. vulgaris, S. aureus, A. niger* and *C. albicans* and the minimum inhibitory concentration was 250 µg for *P. vulgaris,* 500 µg for *E. coli* and *S. aureus,* while it was 750 µg against *B. subtilis* and *C. albicans,* 1000 µg for *A. niger.* The methanol extracts of the stem of *E. tirucalli* showed activity against *B. subtilis, E. coli, E. faecalis, S. aureus* and *C. albicans* and its minimum inhibitory concentration was found to be 500 µg for *E. coli* and *S. aureus,* while it was 750 µg for *B. subtilis, E. faecalis* and 1000µg for *C. albicans.* The petroleum ether and hexane extracts did not show activity against the test organisms. The extracts of higher plants can be very good source of antibiotics[5] against various fungal and bacterial pathogens. Higher plants have also made important contributions in the areas such as cancer therapies. Economically the members of the *Euphorbiaceae* are of considerable importance. Some plants are medicinally valuable such as *Croton, Ricinus, Manihot, Buseus, Euphorbia* and *Emblica*[6]. Antidiarrhoeal activity of *E. humifusa* [7]. Antiviral nature of *E. tirucalli* against Epstein-barr virus[8]. *E. tirucalli* shows poisonous effects on fish[9]. Anti-inflammatory effects of the ethyl acetate extract of *E. humifusa* on human brain tumor cells[10]. Anti-inflammatory actions of *E. splendens*[11]. Antibacterial activity *E. pilulifera* against *M. tuberculosis*[12]. Antibacterial nature of aqueous extracts of *E. tirucalli* against plant pathogenic bacteria such as *Erwinia carotovora, Xanthomonas campestris* and *Pseudomonas solanacearum*[13]. Antiulcer properties of *E. microphylla* in rats[14]. In the present study stem *of E. tirucalli* was extracted in five organic solvents and tested for antimicrobial activity against seven bacteria and three fungi. Among the five organic solvents acetone extracts of the stem of *E. tirucalli* were found to be

effective against test organisms as compared to other solvent extracts, followed by chloroform and methanol. Acetone extracts could control all the organisms such as *B. megaterium, B. subtilis, E. coli, E. faecalis, P. vulgaris, P. aeruginosa, S.aureus*, *A. niger, A. fumigatus* and *C. albicans.* Among the tested microorganisms *B. subtilis, E. coli* and *C. albicans* were found more sensitive than the other organisms.

Table No 2
Inhibitory activity of stem extracts of Euphorbia tirucalli (L.)

solvent extract	Product (µg)	AREA OF INHIBITION ZONE (mm^2)									
		A	B	C	D	E	F	G	H	I	J
	Control	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
	100	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
	250	21.21	21.21	31.43	21.21	21.21	21.21	21.21	12.60	12.60	12.60
Acetone	500	21.21	31.43	**88.00**	21.21	43.21	21.21	21.21	12.60	12.60	31.43
	750	31.43	**71.50**	125.70	43.21	**71.50**	43.21	31.43	21.21	21.21	**56.57**
	1000	**56.57**	**125.70**	**169.70**	**71.50**	**106.07**	**71.50**	**56.57**	31.43	31.43	**88.00**
	100	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
	250	12.60	12.60	12.60	12.60	21.21	12.60	12.60	12.60	12.60	12.60
	500	12.60	12.60	21.21	12.60	43.21	12.60	21.21	12.60	12.60	12.60
chloroform	750	12.60	31.43	**56.57**	12.60	**71.50**	12.60	31.43	12.60	12.60	21.21
	1000	12.60	**56.57**	**88.00**	12.60	**106.07**	12.60	31.43	21.21	12.60	43.21
	100	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
	250	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
	500	12.60	12.60	21.21	12.60	12.60	12.60	43.21	12.60	12.60	12.60
Methanol	750	12.60	21.21	43.21	31.43	12.60	12.60	**71.50**	12.60	12.60	12.60
	1000	12.60	43.21	**56.57**	**56.57**	12.60	12.60	**88.00**	12.60	12.60	31.43
Standards		632	172	148	286	148	226	286	164	184	276

A) Bacillus megaterium B) Bacillus subtilis C) Escherichia coli D) Enterobacter faecalis
E) Proteus vulgaris F) Pseudomonas aeruginosa G) Staphylococcus aureus
H) Asperigillus niger I) Asperigillus fumigatus J) Candida albicans

Standards:
Sterptomycin (10µg/ml) for *E.coli;*
Genatmycin (10µg/ml) for *P.aeruginosa* and *P.vulgaris*; Chloromphenicol (30µg/ml) for *S.aureus;*
Vancomycin (10µg/ml) for *B. subtilis;*
Rifampicin (5µg/ml) for *E. faecalis;*
Kanamycin (10µg/ml) for *B.megaterium;*
Nystatin (10µg/ml) for *Asperigillus niger, A. fumigatus* and *Candida albicans*

CONCLUSION

Phytomedicines are effective in treating most of the infectious diseases mainly skin infections. Most of the secondary metabolites, serve as a plant defence mechanism against microorganisms, insects and herbivores[15]. The tabular reports indicated that the acetone extracts of the stem of *E. tirucalli* were found to be effective against the test organisms as compared to other solvent extracts. Hence the detailed investigations and antimicrobial screening of secondary metabolites from this plant may yield promising antimicrobial agents.

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