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Research Article

EXPLORATION OF ANTIMICROBIAL POTENTIAL OF METHANOL EXTRACT OF STEMS OF *EUPHORBIA NERIIFOLIA*

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ABSTRACT
The antimicrobial efficacy of methanol extract of stem of the plant *Euphorbia neriifolia* (Family: Euphorbiaceae) was evaluated against selected pathogenic bacterial strains (*Staphylococcus aureus* ATC-2245, *Streptococcus aerugenosa* U59, *Escherichia Coli* K88, *Pseudomonas aeruginosa, Salmonella typhi* 12, *Proeus vulgaris* CC-52, *Aspergillus niger* 36 and *Candida albicans*. The antimicrobial activity was evaluated by disc diffusion and micro dilution assay methods. Streptomycin and ampicilin were used as standard antibacterial drugs whilst amphotericin B was used as standard antifungal drug. Results of both assays ensured that the stem possess significant antimicrobial activity in terms of antibacterial and antifungal activity. Results are comparable to that of standard drugs selected. It is also evident from results that methanol extract showed better activity against pathogenic bacteria than fungi.
Keywords: *Euphorbia neriifolia*, Antimicrobial activity, Disc diffusion assay, Minimum inhibitory concentration (MIC)

INTRODUCTION

Herbal medicines have been used since the dawn of civilization to maintain health and to treat disease. There is a tremendous historical legacy in folklore uses of plant preparations in medicines. Scientific studies on plants used in ethnomedicine led to the discovery of many valuable drugs[1]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world[2,3]. The World Health Organization estimated that about 80 % of the world's population still believes in herbal drugs for their primary health care[4]. There are indiscriminate uses of synthetic antimicrobial drugs for the treatment of infectious diseases and as a result drug resistance developed in human beings as well as in plant also[5,6]. Sometimes antibiotics cause adverse reaction like hypersensitivity, immunosuppression and allergic reactions. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from various sources, including medicinal plants[7,8].

Euphorbia neriifolia Linn. (Euphorbiaceae) commonly known as "Sehund or thohar" in Hindi, is found throughout the Deccan Peninsula of India and grows luxuriously around the dry, hilly, rocky areas of North, Central and South India. Ayurveda describes the plant as bitter, pungent, laxative, carminative, improves appetite useful in abdominal troubles, bronchitis, tumors, loss of consciousness, delirium, leucoderma, piles, inflammation, enlargement of spleen, anaemia, ulcers and fever[9,10] . As far as our literature survey could ascertain, no information was available on the antimicrobial activities of the stem of *E. neriifolia*. Therefore, the aim of this current investigation was to explore the antimicrobial potential of methanol extract of stem of *E. neriifolia* against some pathogenic organism.

MATERIALS AND METHODS

Plant Material

The stem of *Euphorbia neriifolia* was collected from the rural region of Midnapore, West Bengal, India. The plant was authenticated by the Botanical Survey of India (BSI), Shibpur (W.B), and India. Air dried whole stem (500 g) were powdered in a mechanical grinder and the powdered materials was extracted by methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure in a rotary vacuum evaporator. The concentrated extract (yield 35.42%) was stored in vacuum desiccators for further use.

Preliminary phytochemical studies

The extracts were subjected to various phytochemical tests to determine the active constituents present in the different crude extract following modified method of Kar et al., 2012[11].

Bacterial and fungal stain

The antimicrobial activity of the methanol extract was screened against two gram positive bacteria such as: *Staphylococcus aureus* (ATC-2245) and *Streptococcus aerugenosa* (U59) and four gram negative bacteria such as: *Escherichia Coli* (K88), *Pseudomonas aeruginosa, Salmonella typhi* (12), *Proeus vulgaris* (CC-52) and two fungi such as: *Aspergillus niger* (36), *Candida albicans.* All strains were preserved in freeze dried state and at 4 °C in stab slant agar [12]. The microorganism's cultures were maintained on nutrient agar (NA) medium for 18 h at 37±1 °C.

Media Preparation and antibacterial Activity

The antimicrobial assay of stem was performed by agar disc diffusion method of methanol extract. A loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotary shaker for 24 h to activate the strain.

Muller Hinton Agar (MHA) (3.8 g/100ml) was weighed and dissolved in 100 ml of distilled water in a sterile conical flask. The medium was sterilized by autoclaving and was allowed to cool at room temperature. The molten Muller Hinton Agar was inoculated with 200 µl of the inoculum and poured into the Petri plate.

For Agar disc diffusion method, the disc (0.6 cm) was saturated with 50 µl of the compound, allowed to dry and was introduced on to the upper layer of the medium with bacteria. Antibiotic paper (streptomycin, ampicillin) discs were used as positive control. These test discs was placed on MHA plate swabbed with the culture of microorganisms. The plates were incubated at 37 °C or overnight**.** For each bacterial strain,

controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiments were carried out in triplicate. The results (mean value n = 3) were recorded by measuring the zone of growth inhibition around the discs[13].

The antifungal assays against yeasts were performed as the antibacterial assays described above with the replacement of SDA as an assay medium and the minimum fungicidal concentrations (MFCs) were recorded. Amphotericin B (Bristol-Myers, Germany) was used as a positive control. Plates were incubated at 35ºC for 24 h (*C. albicans*) and 48 h.

Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube

(Bacteriostatic concentration). According to Haniffa, 2012 method with slight modification; Briefly the broth dilution technique was utilized where the plant extract was prepared to the highest concentration in sterile distilled water and serially diluted (two-fold) to a working concentration using nutrient broth and later inoculated with 0.2 ml suspension of bacterial strains. After 18 hours of incubation at 37º C, the test tubes were observed for turbidity. The least where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value.

Table 1: Phytochemical screening of *Euphorbia neriifolia* extract

Phytochemicals	Alkaloid	Terpenoids	Tannin	Saponin	Steroid	Flavonoid	Phenolic	Tannins	Cardiac glycosides
Euphorbia neriifolia	++	+	++	+++	-	+	-	+	++

Table 2: Antibacterial activity of the methanol extract of *Euphorbia neriifolia* by disc diffusion assay

Organisms	Zone of Inhibition					
	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	Streptomycin (50 µl)	Ampicillin (50 µl)
Staphylococcus aureus	02	08	12	16	20	22
Streptococcus aerugenosa	--	04	06	10	18	16
Escherichia Coli	04	06	10	14	18	16
Pseudomonas aeruginosa	04	08	12	18	22	22
Salmonella typhi	04	06	10	16	21	18
Proteus vulgaris	02	05	08	13	18	16

Values are mean of triplicates experiment

Table 3: Antifungal activity of the methanol extract of *Euphorbia neriifolia* by disc diffusion assay

Organism	Zone of Inhibition				
	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	Amphotericin B
Aspergillus niger	--	02	06	12	18
Candida albicans	--	04	10	14	21

Table 4: MIC and MBC value of Methanol extract of *Euphorbia neriifolia* (MEEN)

Organism	**Extract**	**MEEN**		**Streptomycin**	
		MIC	**MBC**	**MIC**	**MBC**
Bacterial stain	*Staphylococcus aureus*	21.32	5.1×10^{-7}	4.0	2×10^{-8}
	Streptococcus aerugenosa	16.1	6.2×10^{-7}	2.3	3×10^{-9}
	Escherichia Coli	14.23	7.2×10^{-7}	4.5	6×10^{-8}
	Pseudomonas aeruginosa	12.69	5.3×10^{-7}	5.3	3×10^{-9}
	Salmonella typhi	11.36	6.5×10^{-7}	1.3	2×10^{-8}
	Proteus vulgaris	15.6	7.2×10^{-8}	2.6	3×10^{-9}
Fungal stain	*Aspergillus niger*	7.8	5×10^{-7}	1.2	3×10^{-9}
	Candida albicans	4	6×10^{-8}	1.6	3×10^{-9}

Values are mean of triplicates experiment

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the methanol extract of *Euphorbia neriifolia* stem revealed the presence of various bioactive components of which alkaloid, saponin, tannin and cardiac glycosides were the most prominent and the result of phytochemical test has been summarized in table-1. All these phytochemicals possess good antioxidant activities and has been reported to exhibit multiple biological effects including anti-inflammatory, antitumor activities. The results of disc diffusion assay of both methanol and water extracts of the stem of *Euphorbia neriifolia* have been tabulated in table-2 and table-3. It is evident from table-2 that the both methanol extract was found to be active against the bacteria like *Escherichia coli* K88, *Staphylococcus aureus* ATC 2245, *Pseudomonas aeruginosa* and *Salmonella typhi* 12.

The results of disc diffusion assay of the crude extracts were compared with that of standard antibiotic Streptomycin (10µg/disc) and ampicilin (10µg/disc) also recorded. Table-3 indicates that the extract is also potent for its antifungal efficacy. The extracts have shown profound antifungal activity with respect to fungal stains namely *Aspergillus niger* 36, *Candida albicans* and results are comparable to that of standard antifungal agent Amphotericin B (10µg/disc). Among bacteria *Escherichia coli* K88 and among fungi *Pseudomonas aeruginosa* are most susceptible to the extracts. The antibacterial activity and inhibition activity of *E. neriifolia* extracts could be attributed to the chemical compounds. The phytochemical investigations demonstrated the presence of alkaloid, saponin and tannin in methanol extract of *E. neriifolia* [14].

Table-4 represents MIC and MBC/MMC values of the methanol extract against bacterial and fungal strains and results were compared with that of standard antibiotics streptomycin and amphotericin B for bacteria and fungi respectively.

CONCLUSION

From above results it can be concluded that the methanol extract of plant *Euphorbia neriifolia* possess significant antimicrobial activity in term of antibacterial and antifungal

effects. This antimicrobbial property against bacteria and fungi surely is due to presence of some antimicrobial substances in stems. Now our study will be directed to explore the lead compound responsible for aforementioned activity from this plant.

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