Research Article

Evaluation of Antimicrobial and Antibacterial activity of *Euphorbia nivulia*

Yeruva Rosi Reddy[1*], V Haranadha Babu[2]
[1]Asst. professor, SVE Trust college of Pharmacy, Humnabad, Karnataka, India.
[2]Professor, G. Pulla Reddy college of Pharmacy, Mehdipatnam, Hyderabad, Telangana, India.
***Corresponding author's E-mail:** rosi_reddy@yahoo.co.in

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ABSTRACT

The present study was under taken on a medicinal plant, which is known for various pharmacological activities like antiallergic activity, anti-inflammatory and anticancer properties. *Euphorbia nivulia* (Euphorbiaceae) is a plant that is used in traditional medicine for the treatment of asthma, inflammation and for so many disorders. By evaluation of scientific basis for the use of the plant, the antimicrobial activity of whole plant is evaluated. Whole plant extract shows its action against some gram negative and gram positive bacteria and fungi. This study determines about chemical constituents present in the plant. The phytochemical constituents of the dried powdered plant were extracted using aqueous and organic solvents (methanol). The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against both gram negative and gram positive bacteria and fungi using the paper disc diffusion method. Results of the phytochemical studies revealed the presence of tannins, saponins, sesquiterpenes, alkaloids and the extracts were active against both gram positive and gram negative bacteria. The activity of the whole plant extracts were not affected when treated at different temperature ranges (4°C, 30°C, 60°C and 100°C), but was reduced at alkaline pH. Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the test organisms showed that the lowest MIC and the MBC were demonstrated against Salmonella paratyphi, *Bacillus subtilis* and *Salmonella typhi* and the highest MIC and MBC was exhibited against *Staphylococcus aureus*. *Euphorbia nivulia* has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control.

Keywords: *Euphorbia nivulia*, Antimicrobial activity, minimum inhibitory concentration, minimum bactericidal concentration, chemotherapy, infectious disease.

INTRODUCTION

The drug discovery is very important process. At the time of invention of new molecule there is necessity of related fields like medicine, biotechnology, chemistry, pharmacology. Plants remain the most common source of antimicrobial agents, reported to have minimal side effects. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. *Euphorbia nivulia* (commonly called Aakujemudu), family Euphorbiaceae, subfamily Euphorbioideae is a disert plant, evergreen tree and it is very rare plant which is having medicinal values at all over the plant. It is rarely cultivated as popular component of many decoctions used as health remedies.

Latex of this plant used to treat jaundice, dropsy, enlargement of liver & spleen, colic, syphilis, leprosy, applied to hemorrhoids, for bronchitis. Leaf juice was used as purgative.

This plant is also used to treat arthritis, cancer, convulsion, diabetes. Because of its wide usage and availability, this study was set out to investigate the antimicrobial activity of the plant and to determine the effect of temperature and pH on the efficacy of the plant as an antimicrobial agent.

MATERIALS AND METHODS

The whole plant of *Euphorbia nivulia* was collected from the foot hills of Tirumala, Chittoor Dist. Andhra Pradesh, India. The plant species were identified and authenticated by the Botanist Dr. K. Madhava Chetty, Sri Venkateswara University, Titupathi, A.P., India.

Preparation of Extracts

This was carried out as earlier described with slight modifications. The freshly collected whole plants were chopped into pieces and shade dried at room temperature (32-35°C) to constant weight for 5 days. 50g of each of the plant parts were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers. Each of the powdered air-dried plant material was extracted with water and methanol. 25g of each powdered sample was mixed in a conical flask with 100ml of deionised distilled water or organic solvent, plugged, then shaken at 120 rpm for 30 minutes and kept for 24 h. After 24 h, each of the extracts was filtered rapidly through four layers of gauge and then by a more delicate filtration through Whatman no1 filter paper. The resulting filtrates were mixed and then concentrated in a

rotary evaporator and subsequently lyophilized to dryness. The yield of powder was 51% from water extracts, 32% from acetone and 17% from methanol extracts for the whole plant.

Test Organisms

Bacteria and fungal isolates used for this work. They included *Escherichia coli, Proteus mirabilis, Pseudomonas aerugenosa, Salmonella typhi, Salmonella paratyphi, Shigella flexnerri* for gram negative bacteria and *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus pyogenes* for gram positive bacteria, all clinical isolates obtained from the Microbiology Laboratory. Fungal isolates used included *Aspergillus flavus, A.fumigatus, A. niger* and the yeast *Candida albicans* and were laboratory isolates obtained from the Microbiology Laboratory. All the bacterial strains were suspended in nutrient broth and incubated at 37°C for 48 h. Nutrient agar (NA) and Potato dextrose agar (PDA) were used for testing the antibacterial and antifungal activity respectively.

Phytochemical analysis

The freshly prepared extracts were subjected to standard phytochemical analyses to test for the presence of alkaloids, flavonoids, triterepenoids, sterols, and saponins, which were responsible for the biological and pharmacological activities in the respective plant extracts.

Determination of antimicrobial activity

Antimicrobial activity of the aqueous and organic extracts of the plant sample was evaluated by the paper disc diffusion method. For determination of antibacterial activity, bacterial cultures were adjusted to 0.5 McFarland turbidity standard and inoculated onto Nutrient agar (oxoid) plates (diameter: 15cm). For the determination of antimycotic activity, all the fungal isolates and *Candida albicans* were first adjusted to the concentration of 106 cfu/ml. Cultures of *Candida albicans* were suspended in sterile solution of 0.9% normal saline and the spores of the other filamentous fungi were suspended in Tanquay buffer and all the cultures were inoculated onto Sabroud Dextrose Agar plates. Sterile filter paper discs (diameter 6mm for bacteria and 13mm for fungi) impregnated with 100µl of extract dilutions reconstituted in minimum amount of solvent at concentrations of 50 and 100mg/ml were applied over each of the culture plates previously seeded with the 0.5 McFarland and 106 cfu/ml cultures of bacteria and fungi respectively. Bacterial cultures and those of *Candida albicans* were then incubated at 37°C for 18 h while the other fungal cultures were incubated at room temperature (30 – 32°C) for 48 h. Paper discs impregnated with 20µl of a solution of 10mg/ml of ciprofloxacin and cotrimoxazole (for bacteria) and nystatin and amphoteracin B (for fungi) as standard antimicrobials were used for comparison. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disc. For methanol extract three replicate trials were conducted against each organism.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the extracts was estimated for each of the test organisms in triplicates. To 0.5ml of varying concentrations of the extracts, 2ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard for (bacterial isolates) and 106 cfu/ml (for fungal isolates) was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin and cotrimoxazole for bacteria and nystatin and amphoteracin B for fungal isolates). A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then incubated at 37°C for 24 h while tubes containing fungal spore cultures were incubated for 48 h at room temperature (30 – 32°C). After incubation the tubes were then examined for microbial growth by observing for turbidity. To determine the minimum bactericidal concentration (MBC), for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar (for bacteria) and saboraud dextrose agar (for fungi) by streaking. Nutrient agar and saboraud agar only were streaked with the test organisms respectively to serve as control. Plates inoculated with bacteria were then incubated at 37°C for 24 hours while those inoculated with fungi were incubated at room temperature (30 – 32°C) for 48 h. After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration.

RESULTS

Phytochemical constituents present in the plant extract included tannins, saponins, sesquiterpenes, alkaloids. Results of the antimicrobial activity of the plant extracts are shown in Table 1. The result shows organisms. The highest activity (diameter of zone of inhibition 26mm) was demonstrated by the methanol extract of whole plant against *E.coli* while the lowest activity (diameter of zone of inhibition 12mm) was demonstrated by the against *S.paratyphi.* Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are shown in Table 2. The result showed that S.aureus had the highest MIC (14.5 mg/ml) and MBC (17mg/ml), while the lowest MIC of 9 mg/ml was shown by *Salmonella typhi*.

Table 1: Results of the antimicrobial activity of the methanol extracts of *Euphorbia nivulia.*

Zone of Inhibition										
Sl. No	**Name of the Microorganisms**	**75 µg/ml**	**50 µg/ml**	**25 µg/ml**	**10 µg/ml**	**5 µg/ml**	**Cp**	**Ct**	**Am**	**Nys**
1	S.aureus	13mm	10mm	R	R	R	30	18	NA	NA
2	Klebsilla	16mm	13mm	10	R	R	30	20	NA	NA
3	Bacillus	18mm	16mm	14mm	13mm	12mm	29	21	NA	NA
4	E. coli	26mm	20mm	R	R	R	29	11	NA	NA
5	*Proteus mirabilis*	20mm	16mm	R	R	R	30	13	NA	NA
6	*Salmonella typhi*	21mm	20mm	R	R	R	31	14	NA	NA
7	*Salmonella paratyphi*	12mm	10mm	R	R	R	27	5	NA	NA
8	*Pseudomonas aerugenosa*	15mm	13mm	S	S	S	35	25	NA	NA
9	*Shigella flexnerri*	17mm	12mm	S	S	S	32	9	NA	NA
10	*Streptococcus pyogenes*	24mm	20mm	R	R	R	NA	NA	29	30
11	*Aspergillus flavus*	18mm	14mm	S	S	S	NA	NA	26	32
12	*A. fumigatus*	19mm	15mm	S	S	S	NA	NA	25	28
13	*A. niger*	-	-	-	-	-	NA	NA	29	30
14	*Candida albicans*	18mm	14mm	R	R	R	NA	NA	25	26

Note:- S-Sensitive R-Resistant *Cp →ciprofloxacin; Ct → cotrimoxazole; Am → amphoteracin B; → Nys → Nystatin; NA → not applicable*

Table 2: Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanol extracts of *Euphorbia nivulia*

MIC & MBC RESULTS		
Name of the organisms	**MIC (mg/ml)**	**MBC (mg/ml)**
S.aureus	14.5	17
Bacillus	14	20
Klebsiella	13	20
Proteus mirabilis	10	14
Salmonella typhi	9	20
Salmonella paratyphi	9	20
Pseudomonas aerugenosa	10	15
Shigella flexnerri	11.5	15
Streptococcus pyogenes	_	_
Aspergillus flavus	_	_
A. fumigatus	_	_
A. niger	_	_
Candida albicans	_	_

DISCUSSION

Phytochemical constituents such as tannins, saponins, sesquiterpenes, and alkaloids compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores. This may therefore explain the demonstration of antimicrobial activity by the whole plant extracts of *Euphorbia nivulia.* The demonstration of antibacterial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds. This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent. Methanol extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity. The demonstration of antimicrobial activity by water extracts provides the scientific basis for the use of

these plants in the traditional treatment of diseases, since most traditional medicine men use water as their solvent in which the decoctions are prepared. Although the plant is used as a decoction with other plants as skin cleanser, all the plant extracts tested did not show any anti mycotic activity against any of the fungi at the tested concentrations. Cleansing activity may be as a result of their synergy with components from other plants and some other metabolites. Temperature resistance may be an indication that the phytoconstituents can withstand higher temperatures. This also explains the traditional usage of these plant parts where a very high temperature is used to boil them and for a longer period of time. The antibacterial activity of the extracts slightly increased at acidic pH. Increase in activity of phyotoconstituents in the presence of acidic medium has earlier been reported.

CONCLUSION

The demonstration of broad spectrum of antibacterial activity by *Euphorbia nivulia* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of gastrointestinal, urinary tract and wound infections and typhoid fever. The effect of this plant on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

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