

Full Length Research Paper

Phytochemical and antimicrobial studies of leaf extract of *Euphorbia neriifolia*

Kumara swamy M.*, Neeraj Pokharen, Santosh Dahal and Anuradha M.

Padmashree Institute of Management and Sciences, Kommagatta, Kengeri, Bangalore- 560060, India.

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The phytochemical analysis of leaf extracts (chloroform, ethanol, ethyl acetate, butanol and aqueous) of a medicinal plant, *Euphorbia neriifolia* and their antibacterial activities against bacterial isolates, *Staphylococcus aureus*, *Klebsilla pneumonia*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas fluorescens* were investigated. The phytochemical analysis revealed the presence of flavonoids, phlobatannins, saponin, tannins, cardenoloids, phenol, terpenoids. The maximum activity was observed in chloroform extract against *P. vulgaris* with zone of inhibition (8 mm), followed by ethanol extract against *K. pneumonia* (5 mm). The water and ethyl acetate extract exhibited very less activity. This research supports the local use of the leaf of the plant, *E. neriifolia* for wound healing property and other forms of bacterial infections.

Key words: *Euphorbia neriifolia*, phytochemical analysis, antimicrobial activity, medicinal plant.

INTRODUCTION

The medicinal plants are of great interest to human health. Plant based medicines have been a part of traditional healthcare in most parts of the world for thousands of years (Chariandy et al., 1999; Newman et al., 2000). Plants contain numerous biologically active compounds, many of these have been shown to exhibit antimicrobial properties and therefore they were in use as antimicrobial drugs in traditional medicines. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to a report of World Health Organization, more than 80% of world's populations depend on traditional medicine for their primary health care needs. Knowledge of the phytochemicals is desirable not only for the discovery of healthcare products, but also in disclosing new sources of economic materials like alkaloids, tannins, oils, gums etc., (Fransworth, 1966). The systematic screening of plant extracts or plant derived substances still remains an interesting strategy to find new lead compounds in many plant species.

Euphorbia neriifolia Linn. (Euphorbiaceae) grows luxuriously around the dry, rocky, hilly areas of North, Central and South India. It is a herb full of spine,

popularly known as Sehund, Thohar and Milk Hedge. The leaves are thick succulent, 6 to 12 inches long, ovular in shape. *E. neriifolia* leaves are used as aphrodisiac, diuretic and also used in the treatment of bronchitis, bleeding piles and in ano-rectal fistula (Kirtikar and Basu, 1996). The plant is useful in abdominal troubles, bronchitis, tumors, leucoderma, piles, inflammation, enlargement of spleen, anemia, ulcers, fever and in chronic respiratory troubles (Anonymous, 1994). The tribal population of Chattishgarh region uses the milky latex as an ingredient of aphrodisiac mixture (Kirtikar and Basu, 1996; Anonymous, 1952). The aqueous extract of the latex of *E. neriifolia* facilitated the wound healing process as evidenced by increase in tensile strength, DNA content, epithelization and angiogenesis (Rasik et al., 1996). *E. neriifolia* hydroalcoholic extract was found to contain sugar, tannins, flavonoids, alkaloids, triterpenoidal saponin on preliminary phytochemical analysis. Several triterpenoids like glut-5-en-3b-ol, glut-5(10)-en-1-one, taraxerol and b-amyrin has been isolated from powdered plant, stem and leaves of *E. neriifolia* (Anjaneyulu and Ramachandra, 1965). Neriifolione, a triterpene and a new tetracyclic triterpene named as nerifoliene along with euphol were isolated from the latex of *E. neriifolia* (Ilyas et al., 1998; Mallavadhani et al., 2004). Antiquorin have been isolated from ethanol extract example is the occurrence of muscular weakness due to of fresh root of *E. neriifolia* (Ng, 1998). Anti-inflammatory

*Corresponding author. E-mail: swamy.bio@gmail.com.

and analgesic effect of *E. neriifolia* is reported by (Kalpesh et al., 2009). There are reports on the mild CNS depressant, wound healing and immunomodulatory activities of the hydroalcohol leaf extract (Bigoniya and Rana, 2005; Bigoniya and Rana, 2007, 2008). However, to the best of our knowledge, very few reports are available on antibacterial properties of *E. neriifolia*. Hence, in the present study an attempt has been made to evaluate the antibacterial activity of different extracts (chloroform, ethanol, ethyl acetate, butanol and aqueous) of plant leaves against bacterial isolates to ascertain the rationale for its use in traditional medicine. Also, phytochemical screening of the extracts were also carried out with view assess the presence of different phytochemicals in different extracts.

MATERIALS AND METHODS

Plant materials collection

The leaves of *Euforbia neriifolia* were collected from a village, Gummanahalli, Kadur Taluk, Karnataka and planted in the garden of Rishi Herbal Technologies, Bangalore.

Extraction

Leaves of *E. neriifolia* (50 g) were dried in the micro-oven and ground into powder by using an electronic blender. The blended material was transferred into a beaker and 100 ml of chloroform, ethyl acetate, ethanol, butanol and water was separately added and allowed to stand for 72 h and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C.

Phytochemical studies

The methods described by Harborne (1978) were used to test for the presence of the active ingredients in the test sample.

Test for steroids

A 10 ml of leaf extract was evaporated to a dry mass and the mass is dissolved in 0.5 ml of chloroform. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds.

Test for terpenoids

The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids.

Test for tannins

About 1 g of leaf powder was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% FeCl₃ were then added. Production of greenish precipitate indicated the presence of tannins.

Test for flavonoids

A few drops of 1% NH₃ solution is added to the plant leaf extract (0.5 g) in a tube. A yellow colouration is observed if flavonoid compounds are present.

Test for alkaloids

The leaf extract (0.5 g) was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract.

Test for saponins

About 0.5 g of the powdered plant leaf was introduced into a tube containing 5.0 ml of distilled water, the mixture was vigorously shaken for 2 min, formation of froth indicated the presence of saponins.

Bacterial cultures and Media

Bacterial isolates of *Staphylococcus aureus*, *Klebsilla pneumonia*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas fluroscens* were obtained from the Department of Microbiology, Padmashree Diagnostics Pvt Ltd, Bangalore. All bacteria were grown on nutrient agar (NA) at 37°C. For antibacterial assays, bacteria were inoculated into nutrient broth (Himedia, India) and incubated overnight at 37°C.

Antibacterial assay

Antibacterial assay of the extracts was carried out by disc diffusion method (Bauer et al., 1966). Briefly, freshly grown liquid culture of the test pathogens were seeded over the nutrient agar plates with a sterile swab. Sterile filter paper discs of eight mm diameter were soaked with 40 µl of 50 mg/ml the extracts and air dried to evaporate the solvent and the discs were applied over the seeded NA plates at equidistance. The plates were incubated at 37°C for 18 to 24 h. After the incubation period, the plates were observed for a clearance zone around the discs which indicates a positive antibacterial activity of the respective extracts. The clearance zones formed around each disc were measured. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of *E. neriifolia* leaf extracts has revealed the presence of secondary metabolites of therapeutical importance. The major phytochemicals found were phlobatannins, saponins, flavonoids, tannins, phenols, terpenoids and cardenoloids. However, all extracts tested showed the absence of sterols, anthraquinones and cardiac glycosides. Ethyl acetate extract yielded maximum phytochemicals (Table 1). The Anti-inflammatory and analgesic effect of *E. neriifolia* as reported by (Kalpesh

Table 1. Preliminary phytochemical analysis of *E. neriifolia* leaf extracted with different solvents.

Phytochemicals	Chloroform	Ethanol	Ethylacetate	Butanol	Water
Phlobatannins	+	+	+	-	+
Flavonoids	+	+	+	+	+
Saponin	-	-	+	+	-
Phenol	-	-	+	-	-
Tannin	+	+	-	-	-
Terpenoids	-	-	+	-	-
Steroids	-	-	-	-	-
Antraquinones	-	-	-	-	-
Cardenolides	+	-	+	+	-

Note: + Present, -Absent.

Table 2. Antibacterial activity of leaf extracts of *E. neriifolia* at 40 μ l 50 μ g/ml concentration.

Bacteria	Chloroform (mm)	Ethanol (mm)	Ethyl acetate (mm)	Butanol (mm)	Water (mm)
<i>S. aureus</i>	3 \pm 1.2	2 \pm 0.8	0	1 \pm 1.7	0.5 \pm 0.3
<i>K. pneumoniae</i>	3 \pm 0.8	5 \pm 0.4	1 \pm 1.3	1 \pm 1.1	2 \pm 0.4
<i>E. coli</i>	7 \pm 0.5	1 \pm 0.4	1 \pm 1.5	1 \pm 0.7	0
<i>P. vulgaris</i>	8 \pm 0.4	2 \pm 0.9	1 \pm 1.0	1 \pm 0.4	1 \pm 1.6
<i>P. fluorescens</i>	2 \pm 1.0	5 \pm 1.1	0	0.5 \pm 0.5	1 \pm 0.6

The negative control discs were soaked with 40 μ l DMSO and the positive control with 40 μ l of 50 μ g/ml chloromphenicol. Each value represents the mean \pm standard error (SE) of five replicates per treatment in three repeated experiments.

et al., 2009) may be due to the presence of phytochemicals such as terpenoids, tannins, phenolics, saponins and flavonoids (Manach et al., 1996; Alkindele and Adeyemi, 2007). The presence of phlobatannins suggests the diuretic property of the plant (Awoyinka et al., 2007). The wound healing property of this plant can be attributed to the presence of tannins (Kozioc and Marcia, 1998). Elmarrie and Johan (2001) have reported tannin to have antibacterial activity. Tannins and flavonoids are thought to be responsible for antidiarrheal activity (Enzo, 2007). Flavonoids were found in all the extracts and are potent water soluble antioxidants which prevent oxidative cell damage suggesting antiseptic, anticancer, anti-inflammatory effects and mild hypersensitive properties (Okwu, 2004). Phenolic compounds were reported to be very strong antioxidants (Pietta, 2000). Middleton et al. (2000) have reported the antibacterial activity of plant flavonoids. Several authors have reported the antimicrobial activity of crude extracts of various plants (Valsaraj et al., 1997; Oyeleke et al., 2000; Shilpa et al., 2009). Similarly, in *E. neriifolia*, a considerable antibacterial activity was observed. In the present study, all extracts of the leaves of *E. neriifolia* inhibited the growth of most of the bacterial strains tested, but their effectiveness varied. The chloroform and ethanol extracts have shown relatively greater activity

than that of any other extracts at 40 μ l concentration. The maximum inhibition of chloroform extract was observed on *P. vulgaris* (8 \pm mm) and *E. coli* (7 \pm mm) while moderate inhibition was observed on *S. aureus* (3.0 \pm 0.41 mm), *K. pneumoniae* (3.0 \pm 0.41 mm) and *P. fluorescens* (2 \pm 0.44 mm). The ethanol extract have shown greater activity against *K. pneumoniae* (5.0 \pm 0.41 mm) and *P. fluorescens* (5 \pm 0.44 mm). The other extracts did not actively inhibit the growth of the bacteria (Table 2). The observed antibacterial effects on the isolates are believed to be due to the presence of tannins and flavonoids which have been shown to possess antibacterial properties (Cowan, 1999; Draughon, 2004). The results obtained are encouraging as the chloroform and ethanol extracts have shown considerable antibacterial activity against the tested organisms. The screening and scientific evaluation of plant extracts against microbes may provide new antimicrobial substances. Also, plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Hence, the present investigation clearly reveals the antibacterial nature of the plant, *E. neriifolia* and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human systems.

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