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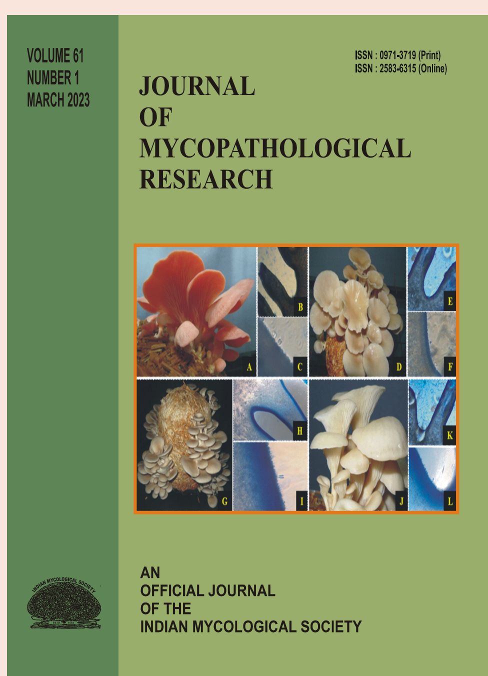

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Evaluation of antioxidant and antimicrobial activities of ethyl acetate extract of *Euphorbia neriifolia* Linn.

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There is very less information available on the *in vitro* antioxidant and antimicrobial activities of *Euphorbia neriifolia* Linn. that belong to the family Euphorbiaceae. Therefore the aim of the current investigation is to analyse the antioxidant and antimicrobial capabilities of the ethyl acetate extract of *E. neriifolia*. The *in vitro* antioxidant of *E. neriifolia* was evaluated by using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method and the antimicrobial capabilities of ethyl acetate extract of leaves and stem of *E. neriifolia* were evaluated against selected pathogenic bacterial strains (*Staphylococcus aureus* (MTCC-3160), *Enterococcus faecalis* (MTCC- 439), *Klebsiella pneumonia* (MTCC- 432), *Escherichia coli* (MTCC- 40), *Aspergillus niger* (MTCC-281) and *Candida albicans* (MTCC-183). The antimicrobial activity was evaluated by well diffusion methods. It is evident from results that ethyl acetate extract showed good activity against pathogenic bacteria and fungi. This study on *E. neriifolia* was carried out to standardize its components. A productive comparability between the physiochemical assessment and antimicrobial activities was observed and the highest range activity of plant extract was noticed. The current investigation clearly indicate the presence of antioxidant and antimicrobial properties in the extract of *E. neriifolia*.

Key words: Antimicrobial activity, Antioxidant activity, *Euphorbia neriifolia*, Well diffusion assay

INTRODUCTION

Thousands of years the traditional knowledge of herbal drugs has been transferred from the old generation to the new generation. The new generation across the world consider plants as a future source of drugs because herbal plant medicines have a strong traditional or conceptual base.

Plants have been used as medicinal substances since the human civilization and mankind came into existence and started to rely on them for healthcare (Upadhyaya *et al.* 2017; Hasan *et al.* 2016). In current scenario approx. 75% population across the world still use herbal or plant based medicines to combat their diseases. Medicine that is based on plants has been used all over the world as traditional healthcare for thousands of years (Newman *et al.*2000; Vadlapudi and Naidu, 2010). According to a study it was estimated that almost 90% of medicines are produced directly from nature (Bigoniya and Rana, 2007). It is possible to treat the various diseases with minimal adverse effects. Not only human beings, but also the entire animal kingdom or living organism fulfil their needs from plants, particularly because of the availability of various bioactive compounds (Shashilata *et al.*2022). World Health Organization estimated that about 80 % of the world's population still believes in herbal drugs for their primary health care. Ethno pharmacology is the study of how personal can get medicines from fungi, animals, plants, shrubs or remaining different naturally resources. *Euphorbia neriifolia* are found gorgeously at the rocky, hilly areas, low humid areas of North, south and central India (Kirtikar, 2006). This plant is widely known as Sehund, Thohar and Milk Hedge amongst the people. The trunk of this plant is 10-15 cm wide, the leaves are thick and juicy, 15-20 cm long, oval in shape. Its leaves are used to treat shortness of

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breath, coughing up thickened mucus, bleeding piles and in ano-rectal fistula (Prashant and Sheetal, 2017). The extract of the latex of *E. neriifolia* promoted the wound healing process as exhibited by expand in tensile strength, DNA content, angiogenesis and epithelization (Janmeda *et al.*2011). The plant is useful in the treatment of bronchitis, stomach troubles, piles, anemia, fever, ulcers, tumours, leucoderma, inflammation, enlargement of spleen, and in chronic respiratory problems (Hernandez *et al.*2003;Chellaiah *et al.*2006). Chhattisgarh's tribes uses the milky latex as an ingredient of aphrodisiac mixture to increase the imunity.

Hydroalcoholic extract of *E. neriifolia* was found to have sugar, flavonoids, alkaloids, tannins, triterpenoidal saponin on preliminary phytochemical analysis. Several triterpenoids like glut-5-en-3b-ol, glut5(10)-en-1-one, taraxerol and b-amyrin has been taken out from powdered plant, stem and leaves of *E. neriifolia* (Bolli and Thorat, 2017). Neriifolione, a triterpene and a new tetracyclic triterpene named as nerifoliene along with euphol were separated from the latex of *E. neriifolia* (Mallavadhani *et al.*2010). There are reports on the mild CNS depressant, wound healing and immunomodulatory activities of the hydroalcohol leaf extract (Vuorela, 2004).

MATERIALS AND METHODS

Plant material collection

The stems and leaves of *Euphorbia neriifolia* were collected in the month of March 2021 from the forest area of Bhopal. The collected aerial parts such as stem and leaves of the plant were in active condition and green. These plant parts were washed in water and then quenched in distilled water and used for research work. Sample was dried at room temperature in a dark place. The leaves and stems were then cut into pieces and left out in a shaded area till completely dry without being contaminated, taking care that the material does not get exposed in direct sunlight. The standards of aerial parts were determined on the basis of accurate parameters suggested by ayurvedic pharmacopeia of India and World Health Organization guidelines. The dried materials were then grounded into coarse powder with the help of an electronic grinder. Then this dried powder was stored in an airtight container in some shaded place at room temperature for extraction and was used for experimental purposes.

Defatting of material

For defatting, the coarse powder of the plant was suspended in petroleum ether for one day at room temperature. After a period of 1 day, the plant material was filtered with the help of spatula, funnel and filter paper so that the impurities can be separated from the materials that dissolved in petroleum ether. The filtered material was uniformly spread on paper to dry and then keep in airtight container.

Chemical reagents

The chemicals used in this research were received from S.R.L. Pvt. Ltd. (Mumbai, India), Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), and SD Fine- Chem. Ltd. (Mumbai, India). All the chemicals and solvents were of analytical grade. The pathogenic microbes used in the current study were obtained from the Microbial Culture Collection, National Centre For Cell Science, Pune, Maharashtra, India.

In-vitro antioxidant activity of ethyl acetate extract by DPPH method

DPPH scavenging activity was measured by the spectrophotometer (Parkhe and Jain, 2018). Stock solution (6 mg in 100 ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 519 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 519 nm. The percentage inhibition of free radical DPPH was calculated from the following equation.

$$\% \text{ Inhibition} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged.

Bacterial and fungal strain

The antimicrobial activity of the ethyl acetate extract was screened against two gram positive bacteria such as *Staphylococcus aureus* (MTCC- 3160), *Escherichia coli* (MTCC- 40), two gram negative bacteria - *Klebsiella pneumonia* (MTCC- 432), *Enterococcus faecalis* (MTCC- 439), and two fungi - *Aspergillus niger* (MTCC-281), *Candida albicans* (MTCC-183), were collected from Bacteriology Unit of the Microbiology Laboratory. All strains were preserved in freeze dried state and at 4 °C in stab slant agar 12. The drugs used for this activity are Ciprofloxacin and Fluconazole.

Media preparation and sterilization of culture media

The antimicrobial assay of aerial parts was performed by well diffusion method of ethyl acetate extract. The agar media 2 g and nutrient media 1.3 g were dissolved in 100 ml. distilled water and then heated in a large conical flask. Dry materials were placed in a flask with the requisite amount of distilled water and heated to completely dissolve the medium. The flask containing the medium was cotton capped and sterilised at 15 lbs/in.^{2} (121°C) for 15 minutes in an autoclave. The media in the flask was immediately poured (20 ml/plate) into sterile Petri dishes on a plane surface after sterilisation. The poured plates were allowed to harden at room temperature before being incubated at 37°C overnight to ensure sterility. Before use, the plates were dried at 50°C for 30 minutes.

Well diffusion method

Broth cultures of those test microorganisms responsive to the phytoextracts employed in this investigation were generated by transferring a loop of culture into sterile nutrient and potato dextrose broth and incubating at 37°C for 24-48 hrs. To generate diffused heavy lawn culture, a loop full of broth was taken and seeded onto sterile nutrient and potato dextrose agar plates using a sterile cotton swab. The well diffusion method was used to determine the antimicrobial activity of the ethyl acetate extract prepared from *E. neriifolia* using standard procedure. There were 3 concentration used which are 25, 50 and 100 mg/ml for extracted phytochemicals in studies. The placement of wells containing antibiotics on the surfaces of agar soon after inoculation with the organism examined is a key component. Inoculums made from undiluted overnight broth cultures should never be used. After incubation at 37°C for 24 h, the plates were evaluated for zones of inhibition around the wells impregnated with a specific drug concentration.

RESULTS AND DISCUSSION

The percentage inhibition of antioxidant activities by DPPH method of both ethyl acetate and ascorbic acid is compared in Table 1 and also shown in graphical representation in Fig.1. The antioxidant activities were not much evident in lower concentrations, but in the highest tested

Table 1: Percentage inhibition of ascorbic acid and ethyl acetate extract of *Euphorbia neriifolia* extract using DPPH method.

Concentration (μg/ml)	Control absorbance	Test Absorbance	% Inhibition	
			Ascorbic acid	*Euphorbia neriifolia* extract
10	0.519	0.469	41.93	9.63
20		0.459	56.45	11.56
40		0.449	61.29	13.48
60		0.419	72.58	19.26
80		0.388	75.8	25.24
100		0.349	80.64	32.75
		IC 50	14.23	176.09

Table 2: Antimicrobial activity of the ethyl acetate extract of *Euphorbia neriifolia* by well diffusion assay against selected microbes.

Name of drug	Microbes	Zone of Inhibition (mm)		
		10 µg/ml	20 µg/ml	30 µg/ml
Ciprofloxacin	*Escherichia coli*	16.5±0.23	21.2±0.10	28.5±0.26
	Klebsiella pneumonia	19.5±0.36	28.4±0.36	36.1±0.15
	Staphylococcus aureus	12±0.94	17±0.57	18±0.74
	Enterococcus faecalis	19±0.5	25±0.94	28±0.5
Fluconazole	*Aspergillus niger*	8±0.74	10±0.5	14±0.5
	Candida albicans	26±0.15	30±0.2	32±0.2

Table 3: Antibacterial activity of *Euphorbia neriifolia* extract against selected microbes

Microbes	Zone of Inhibition		
	25 mg/ml	50 mg/ml	100 mg/ml
Escherichia coli	10±0.5	12±0.5	15±0.47
Klebsiella pneumonia	6±00.47	10±0.74	12±0.74
Staphylococcus aureus	7±0	10±0.47	13±0.5
Enterococcus faecalis	6±0	8±0.57	10±0.86

Table 4: Antifungal activity of *Euphorbia neriifolia* extract against selected microbes

Microbes	Zone of Inhibition		
	25 mg/ml	50 mg/ml	100 mg/ml
Aspergillus niger	6±0	9±0.5	11±0.94
Candida albicans	8±0.57	10±0.94	13±0.74

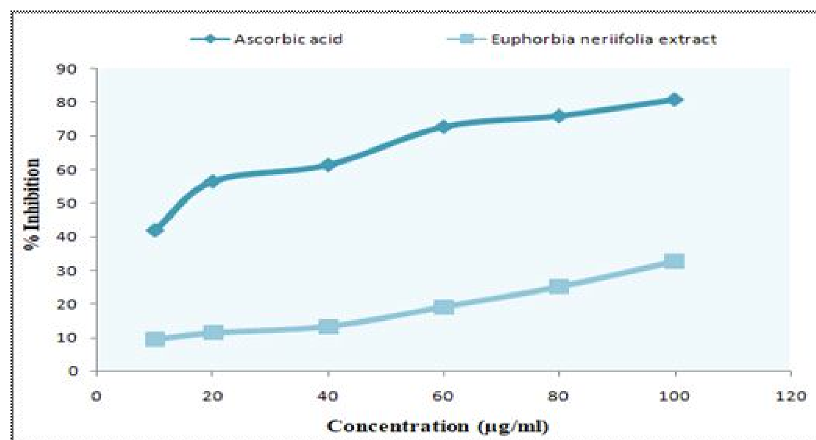

Fig.1: Percentage inhibition of ascorbic acid and ethyl acetate extract of *Euphorbia neriifolia* extract using DPPH method.

concentration inhibition was nearly 50% of the ascorbic acid value. The results of well diffusion assay of both ethyl acetate and water extracts of the aerial part of *E. neriifolia* have been tabulated in Table 3 and Table 4. It is evident from Table 3 that the ethyl acetate extract was active against the bacteria like *Staphylococcus aureus*, *Enterococcus faecal*, *Klebsiella pneumonia* and *Escherichia coli*. The results of well diffusion assay of the extracts were compared with that of standard drugs Ciprofloxacin (10, 20 and 30 μg /ml) also recorded. Results (Table 4) indicate 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*, *Candida albicans* and results are comparable to that of standard antifungal agent Fluconazole (10, 20 and 30 μg /ml).

CONCLUSION

After study of above results it can be concluded that the ethyl acetate extract of plant *E. neriifolia* possess certain *in vitro* antioxidant and antimicrobial activity in terms of antibacterial and antifungal effects. This antimicrobial property against bacteria and fungi would be due to presence of some antimicrobial substances in leaves and stem of this plant. Such screening of these natural organic compounds and identifying active agents is very important so that a remarkable progress can takes place in drugs development.

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