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Evaluation of *in vitro* antimicrobial activity of *Portulaca quadrifida* L.

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SUMMARY

The antimicrobial activity of hexane, methanol and aqueous extracts of *Portulaca quadrifida* (Portulacaceae) was tested by agar gel diffusion method. All the three extracts at a concentration of 100mg/ml showed zone of inhibition ranged from 15-25 mm against 1×10^7 microbial cells. Methanol extract was most effective against both bacteria and fungi. Hexane extract showed moderate where as aqueous extract showed low activity. The largest zone of inhibition against *E.coli* indicates that the extract was simply inhibitory towards this microorganism and *Bacillus subtilis* was most resistant bacteria. All extracts of *Portulaca quadrifida* showed different levels of antifungal activity against *Aspergillus niger* and *Candida albicans*. Tetracyclin and Nystatin were used as standard reference.

Key words : *Portulaca quadrifida*, Antibacterial activity, Antifungal activity

Portulaca quadrifida is a prostrate, mat-forming annual or short-lived perennial herb with much-branched, spreading, articulated, fleshy stems up to 30 cm long or longer, rooting freely from the nodes, often flushed reddish; nodes with a dense whorl of whitish hairs (Kiritikar and Basu, 1933). Vernacular names are small-leaved purslane; single-flowered purslane; ten 0'clock plant; stone-crop; chicken-weed (En). *Portulaca quadrifida* may contain oxalates in toxic quantities which may cause death in live stock. In some soils it also tends to accumulate nitrates and thus should be consumed in moderate quantities (Burkill, 1997). The plant is used in skin diseases and in diseases of the kidneys, bladder and lungs (Chopra and Chopra, 1958). The general uses are as a diuretic, to treat rheumatism and gynecological diseases, as a sedative, analgesic and cardiotonic, to treat fever, disorders of the urinary tract, worm diseases, as a tonic and choleretic, to treat dysentery, and to apply externally to ulcers, eczema and dermatitis (Kokwaro, 1993). The leaves are diuretic, used in dysuria and externally applied in erysipelas. Seeds used as a verminfuge. The aim of present study is the screening of antibacterial and antifungal activity of *Portulaca quadrifida*.

MATERIALS AND METHODS

Plant material:

Whole plants of *Portulaca quadrifida* were collected from rural area of Visakhapatnam district and authenticated by curator of Botany Department, Andhra University, Visakhapatnam. A voucher specimen was deposited in our laboratory. The plant material was shade dried at room temperature until extraction.

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Preparation of extractions:

Hundred grams of shade dried and coarsely powdered plant material was exhaustively extracted for seven hours with hexane (62-65^{0}C) in soxhlet apparatus. The hexane extract was distillated and evaporated under reduced pressure using Rota-Vapor (Heidolph, Heizbad, Laborota 4001, Germany, 2000). The extracted plant material was then air dried, repacked in soxhlet apparatus and exhaustively extracted with methanol (98.8%) until color changed into normal. The methanol extract was filtered, distillated and evaporated under reduced pressure using rota-vapor. The extract was dissolved in dimethyl-sulfoxide to make the final concentration to 100 mg/ml which kept in refrigerator till used (Williams *et al.*, 2003).

Simultaneously, aqueous extract was prepared by infused in distilled water until complete exhaustion. The extract was then filtered using Whatman No.1 filter paper and the filtrate was evaporated in vacuo and dried using rotary evaporator at 60^{0}C (Kandil *et al.*, 1994). The final dried samples were stored in refrigerator.

Microorganisms:

The slants of seven organisms, five bacteria and two fungi were procured from Institute of Microbial Type Culture Collection (MTCC) and Gene Bank , Chandigarh, India. The bacterial strains are *Escherichia coli* (MTCC 2401), *Klebsiella pneumoniae* (MTCC 2405), *Proteus vulgaris* (MTCC 1771), *Bacillus subtilis* (MTCC 2274) and *Staphylococcus aureus* (MTCC 1144) and the fungi are *Aspergillus niger* (MTCC 2594) and *Candida*

albicans (MTCC 2512). They were cultured in laboratory in Petriplates containing nutrient agar for bacterial and potato dextrose agar (Himedia) for fungi as per the guidelines given by MTCC Institute.

Antimicrobial assay:

The most widely used type for identifying antimicrobial activity is the diffusing methods which exploit diffusion of antimicrobial compounds through agar medium to demonstrate inhibition of bacteria and fungi.

The assay was performed using agar disk diffusion method for solvent extracts (Murry *et al.*, 1995 and Sevtap Arikan *et al.*, 2002). A loopful of different tested pathogenic strains were inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotary shaker for 24 hrs to activate the strains of bacteria and in potato dextrose broth for fungi.

The tested strain of bacterial broth culture was inoculated into the 20 ml of nutrient agar medium (Himedia) and care was taken in ensure proper homogenization and poured into Petridishes and allow them to cool under strict aseptic conditions. The above procedure was repeated for fungal strains, except for the media, potato dextrose agar used instead of nutrient agar.

After the medium was solidified a well was made in the Petri plates with the help of a cup borer (5 mm diameter). To determine the potential of the tested hexane, methanol and water extracts were diluted to 100 mg/ml of DMSO solution. Of which 50 µl was introduced into the well and diffusion was allowed for 45 minutes and then they were incubated at 37^0C for bacteria and fungal strains were incubated at 25^0C for 24 hrs for bacterial strains and 36 hrs for fungal strains (Janseen *et al.*, 1987).

After proper incubation of cultures the antimicrobial activity was determined by measuring the diameter of the zone of inhibition around the well by using the Hiantibiotic zone scale-c. The solvents used for reconstituting the extracts for bioassay *i.e.*, DMSO and hexane, methanol and water were analyzed as controls. Growth was determined by turbidity in comparison with the control sample. A zone of inhibition 10 mm excluding diameter of the well was taken as an indicator of active inhibition. The standard antibacterial drug Tetracyclin (30 µg/ml) and antifungal drug Nystatin (30 µg/ml) were used for comparison. For each treatment triplicates were maintained and the mean value is recorded in Table 1.

RESULTS AND DISCUSSION

Table 1 (Graph) shows the antimicrobial activities of hexane, methanol and water extracts of *Portulaca quadrifida* against bacteria (*Escherichia coli ,Klebsiella pneumoniae, Proteus vulgaris, Bacillus subtilis, and Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*). The extracts exhibited remarkable activity at 100 mg/ml concentration. In this investigation the methanol extract of *Portulaca quadrifida* recorded significant antimicrobial activity against all tested bacteria and fungi, while hexane extract

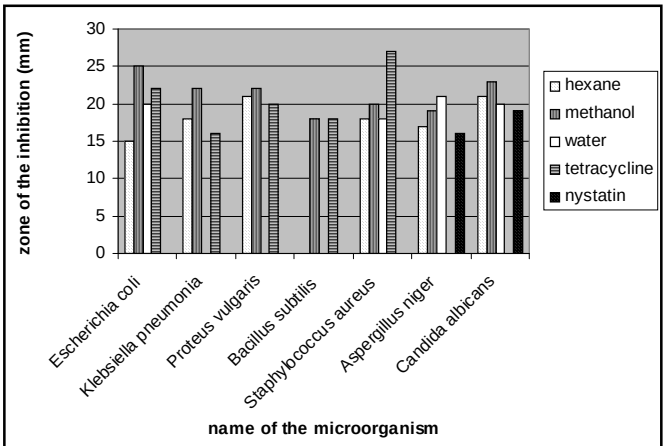

Fig. 1 : Antimicrobial activity of different extracts of *Portulaca quardrifida*

Table 1: Antibacterial and antifungal activity of *Portulaca quadrifida*

Sr. No.	Test oganisms	Zone of Inhibition (mm)				
		Hexane extract 100mg/ml	Methanol extract 100mg/ml	Water extract 100mg/ml	Tetracycline 30 µg/ml	Nystatin 30µg/ml
1.	*Escherichia coli*	15±0.28	25±0.63	20±0.36	22±0.28	--
2.	*Klebsiella pneumoniae*	18±0.54	22±0.36	--	16±0.54	--
3.	*Proteus vulgaris*	21±0.28	22±0.28	--	20±0.28	--
4.	*Bacillus subtilis*	--	18±0.36	--	18±0.49	--
5.	*Staphylococcus aureus*	18±0.49	20±0.54	18±0.63	27±0.28	--
6.	*Aspergillus niger*	17±0.36	19±0.63	21±0.54	--	16±0.36
7.	*Candida albicans*	21±0.28	23±0.36	20±0.36	--	21±0.44

Values are expressed in mean ± SEM of 3 individual experiments.

recorded medium activity. Though methanol extract showed broad spectrum of activity against all tested organisms. *E.coli* was highly sensitive to methanol extract. Hexane extract showed medium activity against all organisms except *Bacillus subtilis. The* aqueous extract presented antibacterial activity against *E.coli* and *Staphylococcus aureus* only. Regarding anti fungal activity all extracts of *Portulaca quadrifida* were sensitive to *Aspergillus niger* and *Candida albicans*. The strongest antifungal activity was observed using the methanol extract. Tetracyclin and Nystatin were used as reference drugs at 30 µg/ml concentration.

The hexane and methanol extracts of *Portulaca quadrifida* showed significant antibacterial activity against Gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris*) and Gram positive bacteria (*Bacillus subtilis, and Staphylococcus aureus*), respectively. The significant and higher antibacterial activity was due to the presence of flavonoids in the plants. Antimicrobial activity of flavonoids is due to the ability to complex with cellular and soluble proteins and to complex bacterial cell walls (Cown, 1999). More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.*, 1996).

The methanol extract of *Portulaca quadrifida* showed highest antifungal activity against *Candida albicans.* It remains the most common infection causing fungus; about 45% of clinical fungal infections are caused by *Candida albicans* (Gupta *et al.*, 2004). The present study showed that methanol extract from *Portulaca quadrifida* had potential anti-*Candida albicans* activity. The antifungal activity may be due attributed to various clinical detectable in its extracts such as saponins (Zhang *et al.*, 2006). The action mechanism of Saponins may lie in damage to membrane and leakage of cellular materials, ultimately leading to cell death (Mshvildadze *et al.*, 2000).

The antimicrobial activity of the *Portulaca quadrifida* against both bacteria and fungi may be indicative of the presence of broad spectrum antibiotic compounds are simply general metabolic toxins in the plant. Since *Portulaca quadrifida* demonstrates activity against the most prevalent bacteria and fungi. Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases. This is the first report of antimicrobial activity found in *Portulaca quadrifida*. Therefore, further detailed study is very much needed for the isolation and identification of active compounds responsible for the antimicrobial activity.

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