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Short Communication Article

Antimicrobial activities of *Kalanchoe pinnata*

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ABSTRACT

Objectives: The present study was aim to evaluate the antimicrobial efficacy of the ethanol extract of the leaves obtained from *Kalanchoe pinnata.*

Methods: The leaves of *K. pinnata* was extracted incorporating the conventional methods of soxhlet extraction using ethanol as menstrum. The dried extract obtained was evaluated for antimicrobial activities incorporating agar dilution and disc diffusion methods over various bacterial strains viz. *Bacillus licheniformis* (MTCC No-429), *Bacillus subtilis* (MTCC No-441), *Escherichia coli* (MTCC No-40), *Pseudomonas aeruginosa* (MTCC No-424), *Staphylococcus aureus* (MTCC No-87), *Proteus vulgaris* (MTCC No-426), *Staphylococcus epidermidis* (MTCC-2639) and *Shigella flexneri* (MTCC No-1457). Minimum inhibitory concentration values were compared with control and zone of inhibition values were compared with standard ciprofloxacin in concentration 100 and 200 µg/ml.

Results & conclusion: The results revealed that the ethanol leaf extract obtained from *K. pinnata* showed significant activity against gram positive bacterial as compared to gram negative bacteria.

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1. Introduction

Kalanchoe pinnata (Family: Crassulaceae) is a succulent perennial plant that grows 3-5 feet tall. Commonly known as 'air plant', it has tall hollow stems, freshly dark green leaves that are distinctively scalloped and trimmed in red, and bell like pendulous flowers. It is used in folk medicine in tropical Africa, tropical America, India, China, and Australia. It is widely used in traditional medicine for the treatment of variety of ailments like anthelmintic, immunosuppressive, hepatoprotective, anti-inflammatory, nephroprotective, anticonvulsant, neuropharmacological and antipyretic. It is well known for its haemostatic and wound healing properties (Kirtikar, 2003; Anonymous, 1997; Nandkarni, 2005),

In the present investigation the ethanolic extract was subjected for study of antimicrobial activity against the different bacterial strains *Bacillus licheniformis* (MTCC No-429), *Bacillus subtilis* (MTCC No-441), *Escherichia coli* (MTCC No-40), *Pseudomonas aeruginosa* (MTCC No-424), *Staphylococcus aureus* (MTCC No-87), *Proteus vulgaris* (MTCC No-426), *Staphylococcus epidermidis* (MTCC-2639) and *Shigella flexneri* (MTCC No-1457) by agar dilution and disc diffusion methods.

2. Material and methods

2.1. Plant material

The leaves of *Kalanchoe pinnata* were collected in the month of February 2015, from the local area of Berhampur, Odisha. The collected aerial part with complete herbarium was authenticated at Department of Pharmacognosy, Royal college of Pharmacy and Health Sciences, Berhampur. The collected leaves of *Kalanchoe pinnata* were washed, shade dried and milled in to coarse powder by mechanical grinder. The powder materials were passed through sieve number 40 and used for

further studies. The bacterial stains were collected from M.T.C.C, Institute of Microbial Technology, Sector-39-A, Chandigarh-160036, India.

2.2. Preparation of extract

The dried coarse powder leaves were extracted in Soxhlet apparatus by using ethanol as extracting solvent for 72 hours. The ethanol extract was concentrated at reduced pressure using rotary evaporator and further subjected for antibacterial activity study. The yield of the extract was found to be 12.11% (w/w).

2.3. Determination of Minimum inhibitory concentration (MIC)

The molten nutrient agar media (Agar oxoid-105%, bacteriological peptone oxoid-1%; beef extract oxoid-0.5%; sodium chloride analar-0.5% and pH 7.2-7.4) was prepared and distributed in Mc cartney bottles, 20 mL each, prior to sterilization. A measured amount of the ethanol extract was added to each bottle in such a manner that the final concentration per mL of the agar medium was 0 (control), 50, 100, 200, 300, 400, 500, 600 and 700 µg/mL. The final mixture was poured individually into 100 mm sterile petriplates.

For uniform diffusion of the drug throughout the medium, the nutrient agar plates containing different concentrations of the drug were refrigerated overnight at 40°C and then dried for 24 hours at 370°C before inoculation. One loopful (loop diameter-2mm) of an overnight grown peptone water culture (Bacterological peptone oxoid-1%, sodium chloride analar-0.5% ,pH 7.4) of each of the test organism at concentration 106 colony forming units (cfu/mL) was placed in all the petriplates marked by checkerboard technique. The spot inoculated plates were incubated at 370°C for 24 hours and then observed for any growth of microorganisms. The minimum concentration of extract which prevent bacterial growth was taken as MIC. The antibacterial growth was observed by formation of bacterial colony or turbidity around the inoculum's spot (Mazumder et al., 2000; Dastidar et al., 2004).

2.4. Determination of Zone of Inhibition by Disc Diffusion method

The antimicrobial activity in terms of zone of inhibition of the ethanol extract was determined against eight different bacterial strains (Table 2) and the results were compared with Ciprofloxacin as standard. All the dilutions for preparation of test and standard drug were done in double distilled sterile water. The nutrient agar plates were prepared and incubated at 370°C for 24 hours and then checked for any sort of contamination. An overnight grown peptone water culture of the bacterial strains to be tested (at concentration 106 colony forming units, cfu/mL) was spread on the solid media plates with a sterile swab. Filter paper discs 6 mm diameter was impregnated with two concentrations each of test and standard drug (Test drug-100 and 200 µg/mL, Standard drug-100 and 200 µg/mL) were placed at the centres of the inoculated plated marked as quadrants and each petriplate receive two concentrations each of standard and test drugs. After refrigerating the plates at 4°C for 2 hours, the plates were incubated at 37±2°C for 24 hours. The antibacterial activity was measured as a diameter in mm of inhibitory zones on the agar plates (Miles et al., 1996; Anonymous, 2003; Mishra et al., 2007). The experiment was repeated in triplicate and average value was written in Table 2.

3. Results and Discussion

The observation of the MIC study has been tabulated in Table 1 and it was found that the minimum inhibitory concentration of the ethanol extract was found to be varying between 50-700 µg/mL, with respect to most of the test bacteria. The MIC of ethanol extract for bacterial strain *Bacillus licheniformis* MTCC No-429 and *Staphylococcus aureus* MTCC No-87 were found to be 400µg/mL, for *Staphylococcus epidermidis* MTCC-2639 was 500µg/mL and for *Pseudomonas aeruginosa* MTCC No-424 was 600 µg/mL. The result of ZOI of the extract and its comparison with standard antibiotics ciprofloxacin (100µg/mL and 200 µg/mL) was recorded in table 2. The ethanolic extract of *Kalanchoe pinnata* showed the significant antibacterial activity in the following decreasing order; *Bacillus licheniformis* > *Staphylococcus aureus* > *Staphylococcus epidermidis* > *Pseudomonas aeruginosa*. From the result of MIC and ZOI values and their competition to that of standard ciprofloxacin, it evident that the ethanol extract is active against gram positive and gram negative bacteria, mostly it active against gram positive bacteria. So, the present investigation offers a scientific support to the ethanomedicinal uses of the plant by the traditional healers.

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Conflict of Interest

Authors report no conflict of interest.

Table 1. Determination of MIC of Ethanol extract of Kalanchoe pinnata against different bacterial strains

SN	Bacteria	Growth on nutrient agar containing different concentrations of ethanol extract (µg/mL)								
		0	50	100	200	300	400	500	600	700
1	*Bacillus licheniformis* (MTCC No-429)	+	+	+	+	+	-	-	-	-
2	*Bacillus subtilis* (MTCC No-441)	+	+	+	+	+	+	-	-	-
3	*Escherichia coli* (MTCC No-40)	+	+	+	+	+	+	+	-	-
4	*Proteus vulgaris* (MTCC No-426)	+	+	+	+	+	+	+	+	+
5	*Pseudomonas aeruginosa* (MTCC No-424)	+	+	+	+	+	+	+	-	-
6	*Staphylococcus aureus* (MTCC No-87)	+	+	+	+	+	-	-	-	-
7	*Staphylococcus epidermidis* (MTCC-2639)	+	+	+	+	+	+	-	-	-
8	*Shigella flexneri* (MTCC No-1457)	+	+	+	+	+	+	-	-	-

'0' stands for plain nutrient agar without the drug serving as control '+' stands for growth and '-' stands for no growth.

Table 2. Determination of zone of inhibition by disc diffusion method

SN	Bacteria	Ethanol extract (µg/mL)		Ciprofloxacin (µg/mL)	
		100	200	100	200
1	*Bacillus licheniformis* (MTCC No-429)	17	21	38	42
2	*Bacillus subtilis* (MTCC No-441)	7	8.9	21	26
3	*Escherichia coli* (MTCC No-40)	6.6	7	14	19
4	*Proteus vulgaris* (MTCC No-426)	6.4	8.7	16	21
5	*Pseudomonas aeruginosa* (MTCC No-424)	8	11	10	27
6	*Staphylococcus aureus* (MTCC No-87)	16.3	18.2	24	29
7	*Staphylococcus epidermidis* (MTCC-2639)	7.2	9	19	25
8	*Shigella flexneri* (MTCC No-1457)	8.2	11.3	17	23

Values are Zone of Inhibition (mm); tests were done in triplicate.

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