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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY OF KALANCHOE PINNATA LEAF

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ABSTRACT

In this study, we have found the in vitro antibacterial activity of chloroform/aqueous extract of leaves of kalanchoe pinnata (lam) pers. The antibacterial activity of the plant leaves evaluated against microbial flora organisms MTCC78pBR322 (in E.Coli), MTCC227 Condid Albican, MTCC265 Rhodococcus Rhodochrous and MTCC2682 Arthrobacter Protophormial. The zome inhibition was measured as the parameter of activity. Amoxycillin 10mg/ml was used as standard compound.The aqueous extract of plant leaves 15 mg/ml of water of injection showed potent 12mm zome inhibition of the bacterial MTCC78pBR322 (in E.Coli) compared with std compound.The leaves extract of chloroform and aqueous at the other bacteria showed less zome inhibition compared than std compound.

Keywords: Extraction, Phytochemical Evaluation and Microbiological assay.

INTRODUCTION

Fig. 1: Photograph of medicinal plant *Kalanchoe pinnata*

Kalanchoe pinnata (syn. Bryophyllum calycinum), Bryophyllum pinnatum, also known as the patharkuchi, Air Plant, Life Plant, Miracle Leaf, Goethe Plant, the Katakataka (Filipino) and also called "Wonder of the World" in the English speaking Caribbean.is a succulent plant native to Madagascar. It is distinctive for the profusion of miniature plantlets that form on the margins of its leaves, a trait it has in common with the other members of the Bryophyllum section of the Kalanchoe genus. The genus was first described by the botanist Michel Adanson in 1763. Reportedly, the name came from the Chinese name for one of the species. This Chinese species is thought to have been either Kalanchoe ceratophylla or Kalanchoe spathulata. The genus Bryophyllum was described by Salisbury in 1806 and the genus Kitchingia was created by Baker in 1881. Kitchingia is now regarded as a synonym for Kalanchoe, whereas some botanists treat Bryophyllum as a separate genus [1]. In these plants, new individuals develop vegetatively as plantlets, also known as bulbils or gemmae, at indents along the leaves. These young plants eventually drop off and take root. No males have been found of one species of this genus which does flower and produce seeds, and it is commonly called, the *Mother of Thousands* [2].

The main plant chemicals found in kalanchoe alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroids, bufadienolides and lipids [3-5] include: arachidic acid, astragalin, behenic acid, beta amyrin, benzenoids, beta-sitosterol, bryophollenone, bryophollone, bryophyllin, bryophyllin A-C, bryophyllol, bryophynol, bryotoxin C, bufadienolides, caffeic acid, campesterol, cardenolides, cinnamic acid, clerosterol, clionasterol, codisterol, coumaric acid, epigallocatechin, ferulic acid, flavonoids, friedelin, glutinol, hentriacontane, isofucosterol, kaempferol, oxalic acid, oxaloacetate, palmitic acid, patuletin, peposterol, phosphoenolpyruvate, protocatechuic acid, pseudotaraxasterol, pyruvate, quercetin, steroids, stigmasterol, succinic acid, syringic acid, taraxerol, and triacontane.

The leaves and bark are bitter tonic, astringent to bowels, analgesics, carminative and useful in diarrhoea and vomiting. Antiulcer [6] activities of the leaf were also reported. Several other biological activities have been reported for *Kalanchoe pinnata* Linn. The plant has hepatoprotective activity and is also used to increase vascular integrity[7], to treat hypertension and kidney stones[8] and to enhance the dropping of umbilical cord of a newly born baby[9]. The leaves of the plant are eaten to control diabetes. They are diuretic, and applied to wounds, boils and bites of insects Leaf juice is used in the treatment of coughs, bronchial affections, blood dysentery, jaundice and gout [10]. *Bryophyllin* compounds have marked anticancer therapeutic values against cancer cells[11]. Immumomodulatory effect [12]. Alcoholic extracts of the leaves showed potent antioxidant activity [13].

MATARIAL AND METHOD

Plant collection –S.D.College of pharmacy and vocational studies Muzaffarnagar

Plant part used-Leaf

Chemical used-Aqueous and chloroform (Anatytical grad)

Apparatus used-Soxhlet Apparatus

Pant Material

We have collected the plant from the rural area and then wash the plant with water for collect the fresh leaf.

Extraction

Extraction with aqeous water

The fresh leaves were cut to reduce size and dried in sun light which is subjected to soxhlation is exhaustively extracted with water for 6 hours.Collect the extract in beaker and evaporate the solvent in hot-air oven at 100^0C to obtained dried content (extract). Stand the dried extract in closed container.

Extraction with chloroform

The fresh leaves were cut to reduce size and dried in sun light which is subjected to soxhlation is exhaustively extracted with chloroform

for 6 hours. Collect the extract in beaker and evaporate the solvent in hot-air oven at 40-50^{0}C to obtained dried content. Stand the dried extract in closed container.

Preparation of extract for in vitro antimicrobial activity

15mg/ml in water for injection

Phytochemical Evaluation

Table 1: Phytochemical Evaluation Test

S. No.	Phytochemical	Test name
1.	Alkaloid	Dragendorff,s reagent Mayer,s reagent Wagner,s reagent Hagar,s reagent Tannic acid test
2.	Saponin glycosides	Heamolysis test
3.	Tannins test	Ferric chloride test
4.	Cardiac glycosides	Cardenolides/bufadienolides

Microbiological Assay: Method A IP1996

I Microbial flora [Obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India]

Bacterium	Catalog number
Escherichia coli	MTCC78pBR322
Condid Albican	MTCC227
Rhodococcus Rhodochrous	MTCC265
Arthrobacter Protophormial	MTCC2682

II Standard Antibiotic Solution

Antibiotic used: SUPRIMOX, Amoxicillin 250mg, Cloxacillin 250mg Vial Suprimox: Inj From Ranbaxy (Rexcel)

Dose: 10mg/ml

Vehicle used: Water for injection

Observation and Calculation

a. Extraction

Table 2: Results of quantitative estimation of Total Extraction

Initial weight	Final Yield	% Yield
50gm	3.98	7.6%

b. Phytochemical evaluation

Phytochemical test

Phytochemical test for the quantitative presence of alkaloids, flavonoides, tannins, saponins and cyanogenic glycosides were measured by methods described by Harborne (1973) and Trease and Evans (1989).

Test for Alkaloids

Mayer's test

Alkaloids give cream color precipitate with Mayer's reagent Potassium mercuric iodide solution.

Dragendorff's test

Alkaloids give reddish brown precipitate with Dragendorff's reagent Potassium bismuth iodide solution.

Wagner's test

Alkaloids give a reddish brown precipitate with Wagner's reagent Solution of iodine in potassium iodide.

Hager's test

Alkaloids give yellow color precipitate with Hager's reagent saturated solution of Picric acid.

Tannic acid test

Alkaloids give buff color precipitate with 10% Tannic acid solution.

Test for Cardiac Glycosides

Kedde's test

Extract the drug with chloroform, evaporate to dryness, add one drop of 90% alcohol and 2 drops of 2% 3,5-dinitrobenzoic acid (3,5-dinitro benzene carboxylic acid-Kedde's reagent) in 90% alcohol. Make alkaline with 20% sodium hydroxide solution. A purple color is produced. The color reaction with 3, 5-diinitrobenzoic acids depends upon the presence of an β- unsaturated-o lactones in the aglycone.

Keller killiani test [test for Deoxy sugars]

Extract the drug with chloroform and evaporate it to dryness. Add 0.4ml of glacial acetic acid containing a trace amount of ferric chloride. Transfer to a small test tube; add carefully 0.5ml of concentrated sulphuric acid by the side of the test tube, blue color appears in the acetic acid layer.

Test for Tannins

Ferric chloride test

Test solution gives blue green color with ferric chloride.

Test for Saponin glycosides

Haemolytic test

Take two test tubes and place in each in 0.2ml of a 10 % solution of blood in normal saline. To one of them, add 0.2 ml of normal saline solution and to the other one add 0.2 ml of the extract of plant prepared by shaking powdered of plant with normal saline solution, and filtering clear. Shake both tubes gently and notice the results.

Microbiological assay

Table 3: Comparison of Solution [Zone of inhibition (mm)] of *Kalanchoe pinnata* reference standard used for microbial Flora organisms

S. No.	Microbial Flora	Solution [Zone of inhibition (mm)]		
		Std (amoxicillin)	KPECH	KPEAQ
1.	MTCC78pBR322 in E.coli	9mm	7mm	12mm
2.	MTCC227 Condid Albican	20mm	11mm	7mm
3.	MTCC265 Rhodococcus Rhodochrous	28mm	7mm	7mm
4.	MTCC2682 Arthrobacter Protophormial	13mm	7mm	7mm

RESULT AND DISCUSSION

Extraction

We have obtained the extract, final yield 2.590 gm and percentage yield 19.3%

Phytochemical evaluation

Table 4: Phytochemical Test Availability of chloroform and aqueous extract

S. No.	Phytochemical	Test name	Availability
1.	Alkaloid	Dragendorff's test	+ -
		Mayer's test	+ -
		Wagner's test	- +
		Hager's test	- -
		Tannic acid test	+ -
2.	Saponin glycosides	Haemolytic test	+ +
3.	Tannins test	Ferric chloride test	+ +
4.	Cardiac glycosides	Kedde's test	+ +
		Keller killiani test	+ +

+ = Present, - = Absent

Microbiological assay

Table 5: Comparison of Solution [Zone of inhibition (mm)] of *Kalanchoe pinnata* reference standard used for microbial Flora organisms

S. No.	Microbial Flora	Solution [Zone of inhibition (mm)]		
		Std (amoxicillin)	KPECH	KPEAQ
1.	MTCC78pBR322 in E.coli	9mm	7mm	12mm
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3.	MTCC265 Rhodococcus Rhodochrous	28mm	7mm	7mm
4.	MTCC2682 Arthrobacter Protophormial	13mm	7mm	7mm

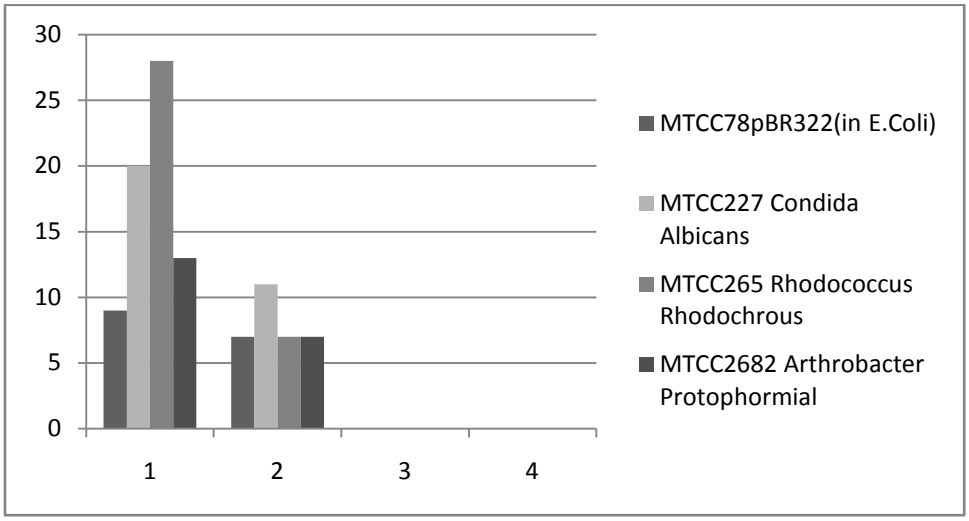

Fig. 2: Comparison between (1) std (2) chloroform

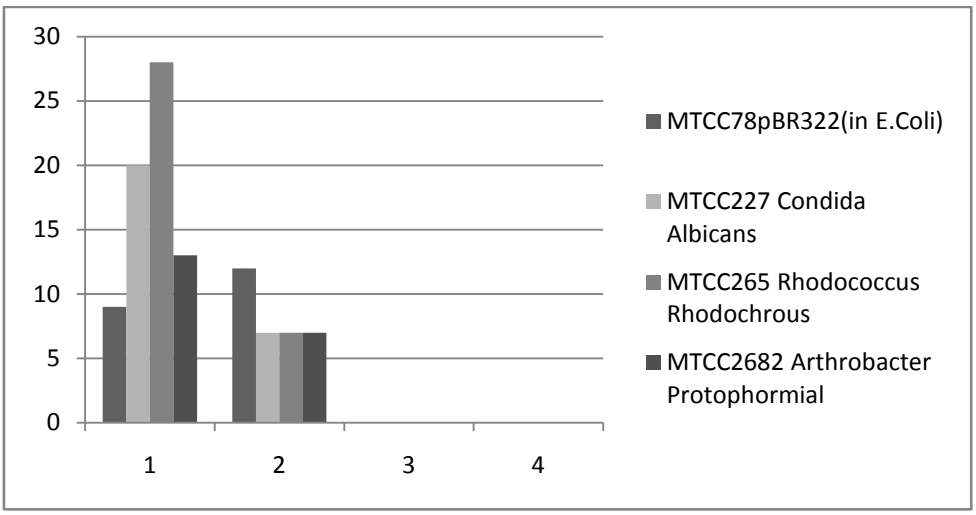

Fig. 3: comparison between (1) std (2) aqueous

CONCLUSION

The findings of the study showed that the aqueous (water) and chloroform extracts of kalanchoe pinnata had successfully inhibit the zones of microorganism. The present study results clearly indicate that aqueous water and chloroform extract of possesses the antibacterial activity of the plant leaves evaluated against microbial flora organisms MTCC78pBR322 in (E.Coli), MTCC227 Condid Albican, MTCC265 Rhodococcus Rhodochrous and MTCC2682 Arthrobacter Protophormial. Further investigation is required to establish its therapeutic effects in antibacterial disease reveal MTCC78pBR322 in (E.Coli), MTCC227 Condid Albican, MTCC265 Rhodococcus Rhodochrous and MTCC2682 Arthrobacter Protophormial.

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