

Original Research Article**IN VITRO EVALUATION OF ANTIBACTERIAL, ANTIFUNGAL AND PHYTOTOXICITY ACTIVITY OF DIFFERENT EXTRACTS OF LEAVES OF KALANCHOE PINNATA**

Yousaf Kamal^{1*}, Bashir Ahmad Ch², Muhammad Uzair², Nadeem Irshad¹, Muhammad Yaseen¹, Iftikhar Hussain³

1. Hamdard Institute of Pharmaceutical Sciences, Islamabad campus, Islamabad-44000, Pakistan
2. Faculty of Pharmacy, Bahauddin Zakariya University, Multan- 60000, Pakistan
3. Faculty of Pharmacy, Hazara University, Mansehra- 21300, Pakistan

ABSTRACT

Background: The emergence of drug resistance in pathogens as well as appearance of undesirable side effects of certain antibiotics has led to the discovery of new antibacterial agents particularly from medicinal plants.

Objective: In this study, we have found the in vitro antibacterial, antifungal and phytotoxicity activity of dichloromethane and methanol extract of leaves of kalanchoe pinnata (lam) pers.

Method: The antibacterial activity of the plant leaves evaluated against microbial flora *Bacillus subtilis* (PTCC 1207), *Bacillus cereus* (PTCC 1247), *Staphylococcus epidermidis* (PTCC 1114) and *Escherichia coli* (PTCC 1047) antifungal activity against *Epidermophyton floccosum*, *Trichophyton mentogrophytes*, *T. rubrum*, *T. simii*, *T. scheenleinii*, *Microsporum canis*, *Pseudallescheria boydii*, *Candida albicans* by using disc diffusion method etc.

Results: Result showed that the tested extracts possess mild or non-significant antimicrobial and antifungal activities. Amoxicillin 10mg/ml was used as standard compound. The leaves extract of dichloromethane and methanol showed less zone inhibition compared than standard compound.

Conclusion: It could be suggested that the dichloromethane and methanol extracts of *Kalanchoe pinnata* leaves possess non-significant antibacterial and anti-fungal activities.

Keywords: *Kalanchoe pinnata*, disc diffusion, Phytotoxicity

***Corresponding Author:** Yousaf Kamal Hamdard Institute of Pharmaceutical Sciences, Islamabad campus, Islamabad-44000, Pakistan

INTRODUCTION

Kalanchoe pinnata (Lam., syn. *Bryophyllum pinnatum*, *B. calycinum*; Local name: Pathorkuchi, Coughpatha; English name: Air plant; Family: Crassulaceae) is an herb found ubiquitously in nature. It has tall hollow stems, fleshy dark green leaves that are distinctly scalloped and trimmed in red, and bell-like pendulous flowers¹. *Kalanchoe pinnata* (*K. pinnata*) has become naturalized in temperate regions of Asia, Australia, New Zealand, West Indies, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia, and Hawaii. It is also widely distributed in the Philippines, where it is known as katakataka or katakataka which means astonishing or remarkable^{1, 2}. The leaves of *K. pinnata* have a variety of uses in the traditional system of medicine in Pakistan. They are eaten for diabetes, diuresis, dissolving kidney stones, respiratory tract infections, as well as applied to wounds, boils, and insect bites¹. The main plant chemicals found in *kalanchoe* alkaloids, triterpenes, glycosides, flavonoids, cardenolides, steroids, bufadienolides and lipids³⁻⁵ include: arachidic acid, astragalol, behenic acid, beta amyryl, benzenoids, beta-sitosterol, bryophollone, 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. Antiulcer⁶ 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 integrity⁷, to treat hypertension⁸ and to enhance the dropping of umbilical cord of a newly born baby⁹. 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 gout¹⁰. *Bryophyllin* compounds have marked anticancer therapeutic values against cancer cells¹¹. Immunomodulatory effect¹² Alcoholic extracts of the leaves showed potent antioxidant activity¹³. Studies on medicinal plant are necessary because there are still plants like *Kalanchoe pinnata* whose medicinal uses have not been fully ascertained in all dimensions. With the emergence of resistant strains of microbes to most existing antibiotics, there is need to challenge them by making continuous frantic search for new more active antimicrobial agents against them. Therefore, present study was designed to scientifically assess the antimicrobial activities of extracts of *Kalanchoe pinnata* on *E.coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* and carry out phytotoxicity activity of the extract.

MATERIAL AND METHODS:

Phytotoxicity activity

Lemna minor was used as a test organism. The experimental conditions included light intensity (9000 Lux), photoperiod (12 hours), incubation condition (28° C, 56 + 1% rh). Phytotoxic activity of the extracts was carried out against the *L. minor* following¹⁴ McLaughlin et al. (1991) and¹⁵ Nisar et al. (2011). Ten grams of leaves of plant was soaked in 90 ml of distilled water and twenty grams of the same plant were soaked in 80 ml of distilled water as two standard solutions of distilled water in conical flasks. The flasks were covered with aluminum foil and allowed to stand at 25 ° C for 48 hrs. The extracts were filtered by Whatman filter paper. The dichloromethane and methanol extracts were further diluted by diluting the initial concentration by a factor of Ghulam Dastagir and Farrukh Hussain. Phytotoxic and insecticidal activity of plants... 84 twenty e.g., (10 ml + 90 ml, 30 ml+70 ml, 50 ml+50 ml, 90 ml +10 ml) plants extracts + distilled water, respectively. The various dilutions were tested for phytotoxicity against *L. minor*. E-medium was prepared by mixing various constituents in 1000 ml distilled water and pH was adjusted between 5.5-6.0 by adding KOH pellets. The constituents and their respective proportions are: 1. Potassium dihydrogen phosphate (0.68gm/L). 2. Potassium nitrate (1.515gm/L). 3. Calcium nitrate (1.180gm/L). 4. Magnesium sulphate (0.492gm/L). 5. Boric acid (0.00286gm/L). 6. Magnesium chloride (0.00362gm/L). 7. Ferric chloride (0.00540gm/L). 8. Zinc sulphate (0.00022gm/L). 9. Copper sulphate (0.00022gm/L) 10. Sodium molybdate. 11. Ethylene diamine tetracetic acid. The E-medium was autoclaved at 121 °C for 15 min. Fifteen (15 mg) of extract was dissolved in 1.5 ml of solvent (Methanol/n-hexane) serving as stock solution. Three sterilized flasks were incubated with 10 µl, 100 µl and 1000 µl of solution pipetted from the stock solution and added to the flasks containing *Lemna* plants. Twenty ml of E-medium was added to each flask. Other flasks were supplemented with E-medium only as control. Plants were examined daily during incubation. Flasks were placed in growth cabinet for 7 days. The number of fronds / flask were counted and recorded on seventh

day. Results were analysed as growth regulation in terms of percentage as described by ¹⁵ (Nisar et al., 2011).

Antimicrobial assay

The antibacterial activity of the methanol extract were determined against *Bacillus subtilis* (PTCC 1207), *Bacillus cereus* (PTCC 1247), *Staphylococcus epidermidis* (PTCC 1114) and *Escherichia coli* (PTCC 1047) using the disc diffusion method. Muller- Hinton agar (MHA) (Oxoid)) was used as bacterial medium. The filter paper discs (6 mm in diameter) were individually impregnated with 10 µL of the stock solution of the extract (4 mg mL⁻¹) and then placed onto the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37°C for 24 h. The diameters of inhibition zones were measured in millimeters. All the tests were performed in duplicate. Gentamicin (30 µg) served as the positive control ¹⁶.

Antifungal Assay

In vitro antifungal screening was performed by disc diffusion assay method (17, 18) where Potato Dextrose Agar (PDA) medium was used for the antifungal activity. Their antifungal activity were tested against six fungal strains at a concentrations of 250 µg/disc, 500 µg/disc for each and the results were compared with griseofulvin (500 µg/disc). The activity was determined after 72 hours of incubation at 37.5°C.

RESULTS:

Anti-bacterial assay:

It has been concluded that tested plant extracts possess no significant anti-bacterial activity against selected strain as in tab.1

Table: 1 Antimicrobial activities of DCE and ME of Kalanchoe pinnata Linn

Test organisms	Zone of inhibition (mm)	
	Dichloromethane extract	Methanolic extract
<i>Bacillus subtilis</i>	–	–
<i>Bacillus cereus</i>	–	–
<i>Staphylococcus epidermidis</i>	–	–
<i>Escherichia coli</i>	1.0±0.33	1.9±0.76

Where,

DCE= Dichloromethane extract, ME= Methanolic extract

Data were represented as mean±SD of triplicate determination and (-): no inhibition

Anti-fungal assay:

Tab showed that KPLM showed non-significant activity against M.canis and F.solani while sample of KPLD showed non-significant activity against A.flavus, M.canis and F.solani.

Table: 2 Antifungal activities of DCE and ME of Kalanchoe pinnata Linn

Test organisms	Zone of inhibition (mm)	
	Dichloromethane extract	Methanolic extract
Epidermophyton floccosum	–	–
Trichophytum mentogrophytes	–	–
T.rubrum	–	–
T.simii	1.0±0.33	1.9±0.76
T.scheonleinii		
Microsporum canis		
Pseudallescheria boydii,		
Candida albicans		

Phytotoxicity assay:

Sample of KPLM showed moderate activity while sample of KPLD showed no activity against the tested plant.

DISCUSSION:

Plants are employed as important source of medication in many traditional medications^{19, 20}

In the present study, the antibacterial test was performed using the disc diffusion method. The antibacterial activities of extracts were assessed against mentioned bacterial strains. In this experiment, both extracts showed non-significant sensitivity to the different strains of bacteria. The antifungal activity was determined results were showing in table.2.

CONCLUSION:

Finally, it could be suggested that the dichloromethane and methanol extracts of Kalanchoe pinnata leaves possess non-significant antibacterial and anti-fungal activities. The experiment was only conducted with certain species of bacteria and fungus as test samples. Therefore, further research is essential to evaluate the sensitivity of plant extract against other species of bacteria, fungi, virus etc. Further studies are however recommended on the plant to determine the pharmaceutical potentialities of the plant as a medicine and to isolate and elucidate the structure of the bioactive compounds.

REFERENCES:

1. Ghani A. Monographs of the recorded medicinal plants. Medicinal Plants of Bangladesh. 2nd ed. Asiatic Society of Bangladesh, p. 271-272 (2003).
2. Descoings B. Illustrated handbook of succulent plants: Crassulaceae. 1st ed. New York: Springer-Verlag Berlin Heidelberg; p. 169 (2003)
3. P.B. Marriage, and D.G. Wilson. Analysis of Organic acids of Bryophyllum pinnatum. Can. J. Biochem. 49: 282-295(1971)
4. K. Gaiind, and R. Gupta. Alkanes, Alkanols, Triterpenes, and Sterols of Kalanchoe Pinnata. Phytochemistry. 11: 1500-1502 (1972)
5. K. Gaiind, and R. Gupta. Identification of waxes from leaves of Kalachoe pinnata. Planta Medica. 23: 193-197(1974)

6. S. Pal, and A.K. Nag Chaudhari. Studies on the Anti-Ulcer activity of a Bryophyllum Pinnatum leaf extract in experimental animals. *J. Ethnopharmacol.* 33: 97-102(1991)
7. Ekpendu TOE, Anyago PU, Ityough D and Akpa F. Nigerian ethnomedicine and medicinal plant flora Benue experience (part III). *Nigerian Journal of Natural Products Medicine.* 4: 13-32 (1998)
8. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *Journal Ethnobiol Ethnomed.*2(45): 1745-1750(2006)
9. Okwu DE and Josiah C: Evaluation of the chemical composition of two Nigerian Medicinal plant. *African Journal Biotechnology.* 5(4): 357-361 (2006)
10. Ghani A. Medicinal plants of Bangladesh, The Asiatic Society of Bangladesh, Dhaka, Second Edition. 382(2003)
11. Unang supratman; Tomoyuki Fujita; Kohki Akiyama; Hideo Hayashi; Akira Murakami; Hirofumi Sakai et al. Anti-tumor promoting activity of Bufadienolides from *Kalanchoe Pinnata* and *K. daigremontiana* multiply *Turifloa*. *Journal of Bioscience, Biotechnology and Biotechnology.* 65(4): 947-949(2001)
12. E.A.Cruz, SAG Da-Silva, MF.Muzitano, PMR Silva, SS Costa, B Rossi Bergmann. Immunomodulatory pretreatment with *Kalanchoe pinnata* extract & its protects mice against fatal anaphylactic shock. *International immunopharmacology.*8, 1616-1621(2008)
13. S. Chandra Mohan, V. Balamurugan, S. Thiripura Salini and R. Rekha. Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe pinnata*. *Journal of Chemical and Pharmaceutical Research.* 4(1):197-202(2012)
14. McLaughlin, J.L., C.J. Chang and D.J. Smith. 1991. Bench-Top bioassays for the discovery of bioactive natural products an update. In: Atta-ur-Rahman (ed.) *Studies in Natural Products Chem.* Elsevier Sci. Publishers, Amsterdam
15. Nisar, M., W.A. Kaleem, M. Qayum, I.K. Marwat, M. Zia-ul-Haq, I. Ali and M.I. Choudhary. 2011. Biological screening of *Zizyphus oxyphylla* EDGEW stem. *Pak. J. Bot.* 43(1): 311-317
16. Lorian V (1996) *Antibiotic in Laboratory Medicine.* Williams & Wilkins, Philadelphia.
17. Bear AW, Kirby WMM, Sherris JC, Turck M. (1966) Antibiotic susceptibility testing by standardized single disc method. *Am. J. Clin. Pathol.* 44: 493-496.
18. Rios JJ, Reico MC, Villar A. (1988) Antimicrobial screening of natural products. *Enthopharmacol.* 23: 127-149
19. Grover JK, Yadav S and Vats V. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol* 2002; 81:81-100
20. Neves JM, Matos C, Moutinho C, Queiroz G and Gomes LR. Ethnopharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (Northern of Portugal). *J Ethnopharmacol* 2009; 124:270-283