REPORT

Antimicrobial activity of *Kalanchoe laciniata*

Maria Manan[1], Liaqat Hussain[1], Hira Ijaz[1] and Muhammad Imran Qadir[2*]
[1]College of Pharmacy, Government College University, Faisalabad, Pakistan
[2]Institute of Molecular Biology & Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

Abstract: This study was conducted to identify antimicrobial potential of *Kalanchoe laciniata*. The plants were extracted with 30-70% aqueous-methanol and n-hexane. The antimicrobial activities were examined using agar well diffusion method against bacteria (*Staphylococcus aureus, Escherichia coli*) and fungi (*Candidaalbicans*). Results showed that *E. coli* were more sensitive than *Staphylococcus aureus* and *Candida albicans*. The largest zone of inhibition (52 mm) was recorded against *E. coli* with the n-hexane extract of *Kalanchoe laciniata.*

Keywords: *Kalanchoe laciniata,* antimicrobial activity, *Escherichia coli, Staphylococcus aureus, Candida albicans*

INTRODUCTION

Diseases caused by bacteria, fungi, viruses and parasites are a big threat to public although there is great progress in medicines (Cosa *et al*., 2006). With the progress in science and technology advances have been done in the fields of medicines with many natural and synthetic drug discoveries (Mahesh and Satish, 2008). Antibiotic was one of the most important discoveries of 20th century, which possesses effectiveness against many bacterial infections. Antibiotic life span is limited. Due to indiscriminate use of antibiotics, multi-drug resistance developed. Antibiotic over prescription problems and misuse are also an issue (Cowan, 1999). Antifungal drugs are costly and fungal species are creating resistant against them. So, new antimicrobials should be discovered for the treatment of various diseases (Diamond, 1993; Perez *et al*., 1990). One way to develop effective and inexpensive drugs, which show antimicrobial activity is to isolate them from the plants which will act for the longer time before the onset of resistance. Plants have been used for centuries for healing purpose (Parihar *et al*., 2010). In the treatment of various diseases in developing countries most of the world population relies on the traditional system of medicines (Islam *et al*., 2008).

The emergence of resistance and tolerance to the existing drugs has created a decreased efficacy of these drugs in use. This problem has been tried to be overcome by increasing the drug delivery to the target site by the use of polymers (Khalid *et al.*, 2009; Hussain *et al.*, 2011) or through nanotechnology (Naz *et al.*, 2012; Ehsan *et al.*, 2012), synthesis of new drugs, either by the use of proteomics (Qadir, 2011; Qadir and Malik, 2011), or synthesis from lactic acid bacteria (Masood *et al.*, 2011), or marine microorganisms (Javed *et al.*, 2011). However, nowadays, the trend is being changed from synthetic drugs to the natural drugs either from plants or microbes to control the diseases. The natural products are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory (Qadir, 2009), hypotensive (Qadir, 2010), hepatoprotective (Ahmad *et al.*, 2012; Ali *et al.*, 2013), hypoglycaemic (Nisa *et al.*, 2009; Qadir and Malik, 2010), amoebicidal (Asif and Qadir, 2011), anti-fertility, cytotoxic, antimicrobial (Amin *et al*., 2012), spasmolytic, bronchodilator (Janbaz *et al.*, 2013a)**,** antioxidant (Janbaz *et al.*, 2012), anti-diarrheal (Janbaz *et al.*, 2013b) and anti-Parkinsonism properties. As a natural product, plant extracts have been emerged as new management tools for the control of different diseases.

Kalanchoe laciniata is species of family Crassulaceae. Traditionally it has been used as astringent, syptic, antiseptic and has been used to treat headache, diabetes, heart discomfort, gastric pain, phthisis, ulcer sores, dysentery, lithiasis and diarrhea. Phytochemical study gives important information about the chemical compound, which helps to decide, whether extract should be further studied for biological activities. *Kalanchoe laciniata* contains saponins, tannins, terpenoids, flavonoids, glycosides and anthraquinones (Manan *et al*., 2015). The ethno-pharmacological based phytochemical research is considered an effective approach for the discovery of novel antimicrobial agents from higher plants (Bigoniya and Rana, 2010). This study was conducted to identify antimicrobial potential of *Kalanchoe laciniata*.

MATERIAL AND METHODS

Plant materials

Kalanchoe laciniata was collected from Faisalabad in July 2013. The plants were identified by Dr. Mansoor

**Corresponding author:* e-mail: mrimranqadir@hotmail.com

Hameed, Taxonomist, Agricultural University, Faisalabad. The plant material was shade dried at 25°C and then ground into coarse powder.

Extractions

Briefly, the coarse powder of *Kalanchoe laciniata* (1000g) was extracted with 30-70% aqueous-methanol and n-hexane for a week at room temperature. The extracts were filtered off through Whatsmann filter paper number 1. Filtrate was evaporated by rotary evaporator at a temperature of 37°C to a thick paste. Pastes were put in Petri dishes for further evaporation. Extracts were weighted and stored in airtight bottles until use.

Microorganisms

The microorganisms used were *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Bacterial cultures were maintained on nutrient agar medium and fungal culture was maintained on sabouraud dextrose agar medium. All cultures were subcultured monthly and stored in incubator.

Screening for antimicrobial activities

Well diffusion method was used to test the antimicrobial activity of the extracts against the bacteria and fungi. On the surface of agar fixed volume of standardized inoculum concentration is spread. Holes (0.5cm) in diameter are punched aseptically with borer. 50µl of plant extract solution was incorporated in each well. Standard disc of Cefoperazone-sulbactum (105µg/ml) and fluconazole solution 150mg/ml were used as positive control. Blanks discs impregnated with solvents (30-70% aqueous-methanol, n-hexane) were used as negative control. 50µl of fluconazole solution (150mg/ml) was also incorporated into the well. The plates were then incubated for 24 hours at 37 C for bacterial cultures and for 48 hours at 37 C for fungal culture. The antimicrobial activity of plant extracts was determined by measuring the diameter of zone of inhibition in millimeter. The experiment was done independently five times and the average zone of inhibition was determined (Valya *et al*., 2009).

STATISTICAL ANALYSIS

Values were given as mean ±SEM and the statistical analysis used was analysis of variance (ANNOVA). $p<0.05$ was considered significant.

RESULTS

During this study *Kalanchoe laciniata* was isolated which was used for the treatment of wound, infectious diseases, abrasion by the tribal people of southern India, Philippines and all over India. Results obtained in the present study showed that both aqueous-methanolic and n-hexane extract of *Kalanchoe laciniata* possess antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Different concentrations of *Kalanchoe laciniata* extracts exhibited significant differences ($p<0.05$) in their efficacy against the tested microbes (table 1). Aqueous-methanolic extract of *Kalanchoe laciniata* showed moderated to good (11-20 mm in diameter zone of inhibition) antibacterial activity at all concentrations against both test bacteria except *Staphylococcus aureus* at 75 mg/ml concentration (5 mm in diameter of zone of inhibition). The n-hexane extract of *Kalanchoe laciniata* showed moderated to very good antibacterial activity (8-52 mm in diameter of zone of inhibition) against the two microorganisms. The largest zone of inhibition (52 mm) was observed against *E. coli* at 300mg/ml concentration with the n-hexane extract of *Kalanchoe laciniata*.

For antifungal activity aqueous-methanolic extract showed efficient antifungal activity at 300 mg/ml concentration, while n-hexane extract showed proficient antifungal activity at 75 mg/ml concentration. Aqueous-methanolic and n-hexane extract exhibited lowest inhibition zone at 75 mg and 300 mg/ml concentration respectively against *Candida albican* (table 1).

DISCUSSION

There is now increase demand for plant based medicines, pharmaceuticals, food supplements, health products and cosmetics (Zachariah *et al*., 2009). Extracts are being obtained from many plants, which possess medicinal properties. Higher plants contain compounds, which are useful as pharmaceuticals and antibiotics (Islam *et al*., 2008). Plants can inhibit pathogens growth or kill them. Recently plants have attracted a lot of attention worldwide for treating bacterial and fungal infectious diseases (Dahanukar *et al*., 2000). Medicinal plants proved to be a reservoir for developing antimicrobial drugs. So plants should be considered as candidates for the development of antimicrobial drugs (Diamond, 1993; Perez *et al*., 1990; Rajendran *et al*., 2009).

Infections are increasing day by day. For bacterial infections antibiotics are the main therapy. The action of antibiotic is rapidly evaded because of development of antibiotic resistance. This resistance created a major clinical problem for the treatment of diseases. Fungal infections with reference to *candida albicans* have become serious health issue over the two decades. Drug toxicity has also created awareness in search of new antimicrobial drugs. There is rich plant diversity. For thousands of years, plant extracts and essential oils have been used in pharmaceuticals, alternative medicines, preservation and natural therapies. For the development of new chemotherapeutic agents plants are important source of effective useful compounds against different microorganisms. Many plants have been investigated for antimicrobial activity. These plants should be studied

Table 1: Zone of inhibition in mm (Mean ± SE) by different extracts of *Kalanchoe laciniata*

Treatment	Strength	Bacterial Strains		Fungal Strain
		S. aureus	*E. coli*	*C. albicans*
Cefoperazone-sulbactum (Antibacterial Standard)	105 µg/disc	24±0.447A	25±0.748A	-
Fluconazole (Antifungal Standard)	150 mg/ml	-	-	21±0.748Z
Aqueous-methanolic extract of *Kalanchoe laciniata*	75 mg/ml	5±0.447B	11±0.447B	11±0.211Y
	150 mg/ml	18±0.447C	18±0.447C	13±0.447Y
	300 mg/ml	20±0.447A	9±0.226B	15±0.447Y
n-Hexane extract of *Kalanchoe laciniata*	75 mg/ml	23±0.447A	30±0.447D	23±0.316Z
	150 mg/ml	13±0.226B	45±0.447E	19±0.316Z
	300 mg/ml	8±0.226B	52±0.447F	12±0.447Y

Similar letter are statistically non-significant (p>0.05)

scientifically which have been used in traditional medicines, so that the quality of health care will be improved. In this study aqueous-methanolic and n-hexane extract of *Kalanchoe laciniata* were investigated for their antimicrobial potential against common microbes.

In vitro studies in this work showed that the *Kalanchoe laciniata* extracts inhibited the microbial (bacterial and fungal) growth but they showed variance in their effectiveness. The antimicrobial activity of these plant extracts had not been previously reviewed. According to antimicrobial screening, all the microorganisms *Staphylococcus aureus, Escherichia coli* and *Candida albicans* were susceptible to plant extracts. Zone of inhibition of *Kalanchoe laciniata* were also comparable with standard drugs (Cefoperazone-sulbactum 105 µg/disc, Fluconazole 150 mg/ml). Zone of inhibition of Aqueous-methanolic extract of *Kalanchoe laciniata* against *Staphylococcus aureus* were larger as compared to *E. coli*. At concentration 300 mg/ml zone of inhibition were 20 mm and 9 mm against *Staphylococcus aureus* and *E. coli* respectively. N-hexane extract of *Kalanchoe laciniata* showed the greatest (52 mm) zone of inhibition against *Escherichia coli*. The n-hexane extract *Kalanchoe laciniata* was more potent for all pathogens as compared to aqueous-methanolic extracts. Cefoperazone-sulbactum (105µg/disc) and fluconazole (150 mg) were the standard drugs used in this study. Cefoperazone-sulbactum zone of inhibition for *Staphylococcus aureus* and *Escherichia coli* were 24 mm and 25 mm and Fluconazole zone of inhibition against *Candida albicans* was 21 mm respectively. The less susceptibility of different microbes at different concentrations of plant extracts have not proved that these bacteria and fungi are resistant but could mean that they need a varied concentration of extract than used or need higher grade solvents for extraction.

CONCLUSION

The present investigation ensures that crude extracts of *Kalanchoe laciniata* possess moderated to very good antimicrobial properties against the selected bacterial (*Escherichia coli*, *Staphylococcus aureus*) and fungal (*Candia albicans*) strains. It was noted that *Escherichia coli* are the most sensitive for the n-hexane extract of *Kalanchoe laciniata* while *Staphylococcus aureus* are the most sensitive for the aqueous-methanolic extract of *Kalanchoe laciniata*.

REFERENCES

Ahmad M, Mahmood Q, Gulzar K, Akhtar MS, Saleem M and Qadir MI (2012). Anti-hyperlipidaemic and hepatoprotective activity of *Dodonaeaviscosa* leaves extracts in alloxan-induced diabetic rabbits (*Oryctolaguscuniculus*). *Pak. Vet. J.*, **32**(1): 50-54.

Ali M, Qadir MI, Saleem M, Janbaz KH, Gul H, Hussain L and Ahmad B (2013). Hepatoprotective potential of *Convolvulus arvensis* against paracetamol-induced hepatotoxicity. *Bangladesh J. Pharmacol.*, **8**: 300-304.

Amin N, Qadir MI, Khan TJ, Abbas G, Ahmad B, Janbaz KH and Ali M (2012). Antibacterial activity of Vacuum liquid chromatography (VLC) isolated fractions of chloroform extracts of seeds of *Achyranthesaspera*. *J. Chem. Soc. Pak.*, **34**(3): 589-592.

Asif MA and Qadir MI (2011). Molecular approaches for development of malarial vaccines. *Rev. Pharmacol.*, **4**: 276-278.

Bigoniya P and Rana AC (2010). Protective Effect of *Wrightiatinctoria* bark triterpenoid fraction on carbon tetrachloride-induced acute rat liver toxicity. *Iran J. Pharma. Thera*., **9**: 55-62.

Cosa P, Vlietink AJ, Berghe DV and Maes L (2006). Anti-infective potential of natural products: How to develop a stronger *in vitro* "proof of concept". *J. Ethnopharmacol.*, **106**: 290-302.

Cowan MM (1999). Plants products as antimicrobial agents. *Cli. Microbe. Review.*, **12**: 564-582.

Dahanukar SA, Kulkarni RA and Rege NN (2000). Pharmacology of Medicinal Plants and Natural Products. *Indian J. Pharmacol.*, **32**: 81-118.

Diamond RD (1993). The growing problem of mycoses in patients infested with human immunodeficiency virus. *Rev. Inf. Dis.*, **13**: 480-486.

Ehsan O, Qadir MI, Malik SA, Abbassi WS and Ahmad B (2012). Efficacy of nanogold-insulin as a hypoglycemic agent. *J. Chem. Soc. Pak.,* **34**(2): 365-370.

Hussain A, Khalid SH, Qadir MI, Massud A, Ali M, Khan IU, Saleem M, Iqbal MS, Asghar S and Gul H (2011). Water Uptake and Drug Release Behaviour of Methyl Methacrylate-co-itaconic acid [P(MMA/IA)] Hydrogels Cross-linked with Methylene Bis-acrylamide. *J. Drug Delvr. Sci. Tech.,* **21**(3): 249-255.

Islam MJ, Barua S, Das S, Khan MS and Ahmed A (2008). Antibacterial activity of some indigenous medicinal plants. *J. Soil. Nature*, **2**: 26-28.

Janbaz KH, Jan A, Qadir MI and Gilani AH (2013a). Spasmolytic, bronchodilator and vasorelaxant activity of methanolic extract of *Tephrosiapurpurea. Acta. Pol. Pharm.*, **79**(2): 261-269.

Janbaz KH, Nizsar U, Ashraf M and Qadir MI (2012). Spasmolytic, bronchodilator and antioxidant activities of *Erythrinasuperosa* Roxb. *Acta. Pol. Pharm.,* **69**(6): 1111-1117.

Janbaz KH, Qadir MI, Jan A and Gilani AH (2013b). Anti-diarrheal activity of methanolic extract of *Tephrosiapurpurea*. *Acta. Pol. Pharm.,* **79**(2): 345-347.

Javed F, Qadir MI, Janbaz KH and Ali M (2011). Novel drugs from marine microorganisms. *Critical Rev. Micro.,* **37**(3): 245-249.

Khalid SH, Qadir MI, Massud A, Ali M and Rasool MH (2009). Effect of degree of cross-linking on swelling and drug release behaviour of poly (methyl methacrylate-co-itaconic acid) [P(MMA/IA)] hydrogels for site specific drug delivery. *J. Drug Delvr. Sci. Tech.,* **19**(6): 413-418.

Mahesh B and Satish S (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J. Agric. Sci*., **4**: 839-843.

MananM, Hussain L, Ijaz H, Nawaz B and Hanif M (2015). Phytochemical screening of different extracts of *Kalanchoe laciniata* (L). *Pak. J. Pharm. Res.,* **1**(2): 58-61.

Masood MI, Qadir MI, Shirazi JH and Khan IU (2011). Beneficial effects of lactic acid bacteria on human beings. *Critical Rev. Micro.*, **37**(1): 91-98.

Naz S, Qadir MI, Ali M and Janbaz KH (2012). Nanotechnology for imaging and drug delivery in cancer. *J. Chem. Soc. Pak.,* **34**(1): 107-111.

Parihar P, Parihar L and Bohra A (2010). *In vitro* antibacterial activity of fronds (leaves) of some important pteridophytes. *J. Microbio. Antimicro.,* **2**: 19-22.

Perez C, Pauli M and Bazerque P (1990). An antibiotic assay by the well agar method. *Acta. Biologiae. Et. Med. Exp.,* **15**: 113-115.

Qadir MI (2009). Medicinal and cosmetological importance of *Aloe vera*. *Int. J. Nat. Ther.,* **2**: 21-26.

Qadir MI (2010). Medicinal values of ginger. *Int. J. Nat. Ther.,* **3**: 19-22.

Qadir MI (2011). Qadirvirtide. *Pak. J. Pharm. Sci.,* **24**(4): 593-595.

Qadir MI and Malik SA (2009). Effect of *Eugenia jambolana* leaves extracts on blood glucose levels of experimental diabetic rabbits. *Pharmacology Online,* **3**: 829-835.

Qadir MI and Malik SA (2010). Anti-diabetic activity of inorganic metals *Eugenia jambolana* Lam. (Myrtaceae) flowers. *Pharmacology Online,* **2**: 979-985.

Qadir MI and Malik SA (2011). Genetic variation in the HR region of the *env* Gene of HIV: A perspective for resistance to HIV fusion inhibitors. *AIDS Res. Hum. Retrovir.,* **27**(1): 57-63.

Rajendran NK and Ramakrishnan J (2009). *In vitro* evaluation of antimicrobial activity of crude extracts of medicinal plants against multi drug resistant pathogens. *Bibad.,* **2**: 97-101.

Valya G, Ragan A and Raju VS (2009). *In vitro* antimicrobial activity of root extract of *Chlorophytum arundinaceum baker*. *Natural Product. Radiance,* **8**: 503-506.

Zachariah SM, Uthumani P and Ramaseshu K (2009). Phytochemistry and antimicrobial screening of stem bark of *Murrayakoenigii* (Linn) Spreng. *The Int. J. Pharm.,* **6**: 12-15.