

Antimicrobial activity of natural compounds from *Kalanchoe crenata* against pathogenic bacteria

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Abstract

Microbial resistance to antibiotics is becoming a source of challenge and concern to public health has led to the evaluation of alternative, effective and affordable substitutes are essential if bacterial infections are to be properly controlled. *Kalanchoe crenata* (Crassulaceae) is a medicinal herb present throughout Bangladesh and is sources of phytochemicals which are able to initiate different biological activities including antimicrobials. Antibacterial potency of the natural compounds of *Kalanchoe crenata* was assessed using five human pathogenic bacteria (gram-negative and gram-positive by disc diffusion and agar well diffusion methods. All the test bacteria were susceptible to these natural compounds. There was no viable microorganism in the initial inoculums after five expositions in the natural compounds of *Kalanchoe crenata*. Our study reflects that these natural compounds obtained from *Kalanchoe crenata* shown strong antibacterial activity and can be serve as a good source for the invention of new therapeutic agents to kill pathogenic bacteria.

Introduction

Infectious diseases are considered a major threat to human health and their complications are continuously increasing throughout the world mainly due drug resistance to human pathogenic bacteria has been commonly reported from all over the world [1-5]. However, the situation is become one of the major problems of humanity in developing as well as developed countries due to indiscriminate use of antimicrobials [6,7]. Traditional medicines play an important role in health services around the World. About three-quarters of the world population relies on plants and plant extracts for healthcare [7]. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Traditional medicine is an important source of potentially useful new compounds for the development of antimicrobial agents [8]. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics such as hypersensitivity, allergic reactions, immunosuppression and are major burning global issues in treating infectious diseases [9]. Although pharmaceutical industries had produced considerable number of commercial antibiotics time to time but resistance in pathogens towards these drugs too has increased at high rate and multi-drug resistant microorganisms have exacerbated the situation [10]. In the present scenario, there is an urgent and continuous need of exploration and development of cheaper and cost-effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active natural compounds from plant species used in herbal medicines [11]. *Kalanchoe pinnata* is generally known as patharkuchi is a succulent plant found in Bangladesh having erected perennial glabrous herb with woody stem and thick, containing succulent leaves with adventitious roots, crenate edges, regularly with foliar buds and developed in nurseries everywhere throughout the nation [12]. The plant contains chemical constituents as alkaloids, triterpenes, glycosides, flavonoids,

cardienolides, steroids, bufadienolides and lipids [12-14]. In the ethno-pharmacological properties, the leaves of this plant have been represented to exhibit anti-ulcer, mitigating and moderating, relieving pain, extraordinary adversary of histamine and anti-allergic activity. *Kalanchoe crenata* extracts are used in ethnomedicine for the treatment of earache, burns, abscesses, insect bites, whitlow, diarrhea and cithiasis [15]. It also used for diabetes, dissolving kidney stones, respiratory tract infections, as well as applied to wounds, boils, and insect bites [16]. *Kalanchoe crenata* has a high phytotherapeutic potential, as showed up by its mitigating, antileishmanial, hepatoprotective, immunomodulatory properties, antithrombotic sway and implemented for cardiovascular treatment [17-19], however, no information was found regarding the pharmacological and phytochemical evaluation. The aim of the study was to clarify the bioactive compounds from *Kalanchoe crenata* to scientifically assess the antimicrobial activities on *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*.

Materials and methods

Plant materials

The *Kalanchoe crenata* plant was collected from the northeast part of Bangladesh. After its identification by Professor Dr. Abdullah

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Key words: bioactive compounds, *Kalanchoe crenata*, antimicrobial activities, microbial resistance

Received: August 19, 2019; **Accepted:** August 27, 2019; **Published:** August 29, 2019

Al Mamun, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh, a voucher specimen (Ref. GEB090320164/1) was submitted to the Laboratory of Alternative Medicine and Natural Product Research, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh.

Sample preparation

The plant part of *Kalanchoe crenata* (leaf) was separately shade dried, finely powdered using a blender, and subjected to extraction of flavonoids in following the method as described elsewhere with some modifications [20-23]. Briefly, two hundred grams of each finely powdered sample was Soxhlet extracted with 80% hot methanol (1000 ml) on a water bath for 24 h and filtered. Filtrate was re-extracted successively with petroleum ether, ethyl ether and ethyl acetate using separating funnel. Petroleum ether fractions were discarded as being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions were analyzed for free and bound flavonoids, respectively. Ethyl acetate fraction of each of the samples was hydrolyzed by refluxing with 7% H₂SO₄ for 2 h for removal of bounded sugars from the flavonoids. Resulting mixture was filtered and filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract thus obtained was washed with distilled water to neutrality. Ethyl ether and ethyl acetate fractions flavonoids were dried and weighed. The extracts were stored at 4°C for farther used and were re-suspended in their respective.

Total flavonoids determination

Total flavonoids content of each extract was determined by aluminum chloride as described elsewhere [with some modifications [20-23]. Briefly, plant extracts (0.5 ml of 1:10 g/ml) were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer, and quercetin was used as a standard for calibration curve. Total flavonoids values are expressed in terms of mg equal quercetin in 1 g powder.

Collection of bacterial isolates

Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-Negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*) were obtained from MAG Osmani Medical College, Sylhet, Bangladesh and identified at the microbiology laboratory, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology.

Test solution preparation

For preparation of test solution of appropriate concentration, natural compounds of *Kalanchoe crenata* were solubilized to autoclaved distilled water and diluted to desired concentration. Precautions were taken during the operation for not to contaminate the culture with foreign contaminants. The test solution was poured into the well using a micropipette. The plates were kept in sterile condition for 30 minutes for diffusion of the test solution to the surrounding media. Addition of the test solution to the plates then inverted into incubator at 37°C and then after 24 hours, we examined of each plate. The circular inhibition zones were formed around the well on the surface. The diameters of the complete zones of inhibition were measured using a ruler [24].

Preparation of mueller-hinton agar medium for assessment of antimicrobial potential

For routine susceptibility testing of non-fastidious bacteria Mueller-Hinton agar is considered to be the best. Medium formulations only that have been tested according to, and that meet the acceptance limits describe in National Committee for Clinical laboratory Standards (NCCLS) document protocols for evaluating dehydrate [24]. Mueller-Hinton agar was prepared according to the manufacturer's instructions from a commercially available dehydrated base.

Evaluation of antimicrobial potential of *Kalanchoe crenata* natural compounds

For *in vitro* investigation of antimicrobial activity of natural compounds of *Kalanchoe crenata* against selected isolates, disc diffusion method [25] and agar well diffusion method [26] were applied. The accuracy and reproducibility of this test are dependent on maintaining a set of standards recommended by the NCCLS [24]. All the identified isolates were subjected to various concentrations of natural compounds of *Kalanchoe crenata* for the evaluation of antibiotic activity pattern.

Agar well diffusion method

In agar well diffusion test, the natural compounds of *Kalanchoe crenata* were allowed to diffuse out into the medium. The antimicrobials present in the natural compounds of *Kalanchoe crenata* interact with the freshly seeded test organisms. This interaction results in a uniform circular zone of inhibition zone was measured in millimeters. From an agar plate of pure culture, 3-5 colonies of the same morphological type were selected. The top of each colony was touched with a loop and the growth was transferred into a tube containing 4-5 ml of Nutrient broth. The broth culture was then incubated at 37° C to attain the preferable turbidity. Sterile cotton swab was dipped into the suspension. The excess fluid was removed by pushing and rotating the swab firmly against the wall, just above the fluid level. The entire dried surface of Mueller-Hinton agar plate was streaked by the swab 2-3 times. That results an even distribution of the inoculums over the entire surface [26].

Disc diffusion method

Antimicrobial activities of the natural compounds of *Kalanchoe crenata* were determined by using the disc diffusion method [24]. The bacterial culture was streaked on Mueller Hinton Agar plate. Blank discs containing the natural compounds of *Kalanchoe crenata* were then placed on the inoculated plate surface and incubated at 37°C for 24 hours. Antimicrobial activity was determined by measuring the diameter of zones of inhibition produced after incubation [24]. All samples of dry residue were dissolved in autoclaved distilled water. The discs were impregnated with the natural compounds of *Kalanchoe crenata* and placed aseptically on the inoculated agar.

Statistical analysis

We used analysis of variance with an F-test, followed by a t-test. *P* values less than 0.05 were considered significant. The data are presented as mean ± standard deviation values of independent replicates.

Results

In the present study, five bacteria (gram-negative and gram-positive bacteria) were used. The antibacterial assays were performed by disc diffusion and agar-well diffusion methods. Therefore, it

could be qualified and quantified by inhibition zone diameters. The bacteria susceptibility to the natural compounds of *Kalanchoe crenata* on the basis of inhibition zone diameters varied according to the microorganism and the results are reported in table 1. There is a significant variation in the diameters of inhibition zone values of the natural compounds of *Kalanchoe crenata* (Table 1).

The results of disc diffusion assay the natural compounds of *Kalanchoe pinnata* showed significant antimicrobial activity that ranged from 10-22 mm zone of inhibition in diameter and highest activity was shown at the concentration of 50 µg/ml against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* (Table 2). These natural compounds did not show any notable antimicrobial activity against *E. coli*, *P. aeruginosa* and *K. pneumoniae* at the concentration 10 µg/ml (Table 3). In the agar well diffusion method the minimum bactericidal concentration of the natural compounds of *Kalanchoe crenata* was investigated and it was showed that the result varied according to the microorganism (Table 2). The natural compounds of *K. pinnata* showed moderate antimicrobial activity in agar well diffusion method and antimicrobial activity ranged from 8-19 mm zone of inhibition in diameter (Table 4) in which highest activity was shown at the concentration of 50 µg/ml against *P. aeruginosa* and *K. pneumoniae* (Table 2). These compounds did not show any antimicrobial activity against *E. coli*, and *K. pneumoniae* at the concentration of 10 µg/ml (Table 3). The results showed that after 4 h exposition into the natural compounds of *Kalanchoe crenata*, there was no viable microorganism in the initial inoculums (Table 5).

Table 1. Inhibition zone diameters (mm) recorder in disc diffusion method using natural compounds of *Kalanchoe crenata*

Microorganisms (Bacteria)	Natural compounds of <i>Kalanchoe crenata</i>
<i>Staphylococcus aureus</i>	22 ± 0.62
<i>Bacillus subtilis</i>	20 ± 1.12
<i>Klebsiella pneumoniae</i>	18 ± 0.73
<i>Pseudomonas aeruginosa</i>	15 ± 0.94
<i>Escherichia coli</i>	10 ± 0.45

Table 2. Bacteriostatic (-) and bactericidal (+) effects of natural compounds of *Kalanchoe crenata*

Bacteria	MIC (µg/ml)	MBC (µg/ml)	Effects
<i>Staphylococcus aureus</i>	50	50	+
<i>Bacillus subtilis</i>	50	50	+
<i>Klebsiella pneumoniae</i>	50	50	+
<i>Pseudomonas aeruginosa</i>	50	50	+
<i>Escherichia coli</i>	50	50	+

Table 3. Bacteriostatic (-) and bactericidal (+) effects of natural compounds of *Kalanchoe crenata*

Bacteria	MIC (µg/ml)	MBC (µg/ml)	Effects
<i>Staphylococcus aureus</i>	10	10	-
<i>Bacillus subtilis</i>	10	10	-
<i>Klebsiella pneumoniae</i>	10	10	-
<i>Pseudomonas aeruginosa</i>	10	10	-
<i>Escherichia coli</i>	10	10	-

Table 4. Inhibition zone diameters (mm) recorder in agar well diffusion assay using natural compounds of *Kalanchoe crenata*

Microorganisms (Bacteria)	Natural compounds of <i>Kalanchoe crenata</i>
<i>Staphylococcus aureus</i>	16 ± 1.38
<i>Bacillus subtilis</i>	19 ± 1.21
<i>Klebsiella pneumoniae</i>	13 ± 0.65
<i>Pseudomonas aeruginosa</i>	10 ± 0.75
<i>Escherichia coli</i>	8 ± 0.68

Table 5. Viability of microorganisms after 5 h exposure of natural compounds of *Kalanchoe crenata*

Bacteria	Time (kill h)					
	0 h	1 h	2 h	3 h	4 h	5 h
<i>Staphylococcus aureus</i>	+ (uc)	+ (uc)	28 ± 1.21	10 ± 0.78	3 ± 0.32	-
<i>Bacillus subtilis</i>	+ (uc)	+ (uc)	26 ± 0.56	7 ± 0.63	-	-
<i>Klebsiella pneumoniae</i>	+ (uc)	+ (uc)	25 ± 0.98	9 ± 0.53	3 ± 0.45	-
<i>Pseudomonas aeruginosa</i>	+ (uc)	+ (uc)	20 ± 0.58	6 ± 0.54	-	-
<i>Escherichia coli</i>	+ (uc)	+ (uc)	21 ± 0.75	4 ± 0.34	-	-

Discussion

Phytochemicals are secondary metabolites to exhibit distinct biologically active and pharmacological effects on microorganisms. Antimicrobial properties of medicinal plants are progressively detailed from various pieces of the world. The World Health Organization evaluates that plant extracts or their active constituents are utilized as ethnic drug in traditional treatments of eighty percent the total population. There are concerning reports of microbial resistance to several antibiotics is becoming a source of challenge to public health.

In case of the increasing of antimicrobial drug resistances, alternative, effective and affordable substitutes are essential to proper control bacterial infections. *Kalanchoe crenata* is a succulent plant is wealthy in alkaloids, triterpenes, glycosides, flavonoids, steroids and lipids, a series of vitamins, macro elements; calcium, potassium phosphorus, microelements; iron, zinc, ascorbic acid, riboflavin, thiamine [27]. In this study revealed that the natural compounds of *Kalanchoe crenata* can play an important role in fighting against the bacterial resistance. The natural compounds of *Kalanchoe crenata* show strong antimicrobial activity against most of the tested bacterial strains. The outcomes were compared with negative control. The studies on disk diffusion method suggest potential activity of the natural compounds of *Kalanchoe crenata* against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* but there is less activity found on *Escherichia coli* shows clear inhibition zones, bactericide effects are visible and zones diameter is quite impressive. The other bacteria show moderate effects along with bacteriostatic actions. In well diffusion method, the natural compounds of *Kalanchoe crenata* show moderate antimicrobial activity compared to control group. The presence of different phytochemicals with biological activity can be of valuable therapeutic index. Several evidences showed that methanolic extracts of *Kalanchoe crenata* leaves have antimicrobial activities [28]. These reports are matched with our findings with some variations. These outcomes support the ethno medicinal claim that this plant displayed potent antimicrobial action. So, the natural compounds of *Kalanchoe crenata* might be utilized in future to cure the microbial infections.

Conclusions

Kalanchoe crenata is a highly therapeutically potential plant to combat microbial infections. The present study gathered significant knowledge on the growing research towards developing new antimicrobial agents. It will be further investigated having more advanced methods and strict protocols to determine further important activities of the *Kalanchoe crenata* and hopefully against infectious diseases.

Competing of interest

The authors declare that they have no competing interests

Acknowledgement

This research work was supported by the Research Grant-2014, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh.

Reference

- Irenji N, Pillai SKG, West-Jones JS (2018) Serious life-threatening multifocal infection in a child, caused by panton-valentine leucocidin producing *Staphylococcus aureus* (PVL-MSSA). *BMJ Case Rep* bcr-2017-222138. [[Crossref](#)]
- Piddock KJV Wise R (1989) Mechanisms of resistance to quinolones and clinical perspective. *J Antimicrob Chemother* 23: 475-483. [[Crossref](#)]
- Singh M, Chaudhry MA, Yadava JNS, Sanyal SC (1992) The spectrum of antibiotic resistance in human and veterinary isolates of *Escherichia coli* collected from 1984-1986 in Northern India. *J Antimicrob Chemother* 29: 159-168. [[Crossref](#)]
- Robin EH, Anril W, Alexander M, Loeto M, Keith K (1998) Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and *Haemophilus influenzae* Type b in children under 5 years of age in Botswana. *Int J Infect Dis* 3: 18-25. [[Crossref](#)]
- Davis J (1994) Inactivation of antibiotic and the dissemination of resistance genes. *Science* 264: 375-382. [[Crossref](#)]
- World Health Organization (2014) Antimicrobial resistance. Antimicrob Resist Glob Rep surveillance. Geneva, *WHO Library Cataloguing-in-Publication Data*: 1-232.
- Abreu AC, McBain AJ, Simões M (2012) Plants as sources of new antimicrobials and resistance-modifying agents. *Nat Prod Rep* 29: 1007-1021. [[Crossref](#)]
- Redo MC, Rios JC, Villar A (1989) A review of some antimicrobial compounds isolated from medicinal plants. *Phytotherapy Res* 3: 117-125.
- Schinor EC, Salvador MJ, Ito IY, Dias DA (2007) Evaluation of the antimicrobial activity of crude extracts and isolated constituents from *Chresta scapigera*. *Brazilian J Microbiol* 38: 145-149.
- Nino J, Navaez DM, Mosquera OM, Correa YM (2006) Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and two Rubiaceae plants from Colombian biodiversity. *Brazilian J Microbiol* 37: 566-570.
- Essawi T and Srour M (2000) Screening of some Palestinian medicinal plants for antibacterial activity. *J Ethnopharmacol* 46: 343-334. [[Crossref](#)]
- Saleem A, Nasir S, Rasool N, Bokhari T H, Rizwan K, et al. (2015) In vitro antimicrobial and haemolytic studies of *Kalanchoe pinnata* and *Callistemon viminalis*. *Int J Chem Biochem Sci* 7: 29-34.
- Gaind K, Gupta R (1972) Alkanes, Alkanols, Triterpenes, and Sterols of *Kalanchoe Pinnata*. *Phytochemistry* 11: 1500-1502.
- Marriage PB, Wilson DG (1971) Analysis of Organic acids of *Bryophyllum pinnatum*. *Ca J Biochem* 49: 282-95.
- Okwu DE, Nnamdi FU (2011) Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial Activity. *J Chem Pharm Res* 3: 1-10.
- Ghani A (2003) Monographs of the recorded medicinal plants. Medicinal Plants of Bangladesh. (2nd Edn.) Asiatic Society of Bangladesh: 271-272.
- Yadav NP, Dixit VK (2003) Hepatoprotective activity of leaves of *Kalanchoe pinnata*. *J Ethnopharmacol* 33: 197-202. [[Crossref](#)]
- Ekpendu TOE, Anyago PUItyough D, Akpa F (1998) Nigerian ethnomedicine and medicinal plant flora- Benue experience (Part III). *Nigerian journal of natural products and Medicines* 4: 13-32.
- Jessica B, Satish S (2008) Antimicrobial Activity of Some Important Medicinal plant against Plant and Human Pathogens. *World Journal of Agricultural Sciences* 4: 839-883.
- Al Mamun MA, Islam K, Alam MJ, Khatun A, Alam MM, et al. (2015) Flavonoids Isolated from *Tridax procumbens* (TPF) Inhibit Osteoclasts Differentiation and Bone Resorption. *Biol Res* 48: 51. [[Crossref](#)]
- Al Mamun MA, Hosen MJ, Islam K, Khatun A, Alam MM, et al. (2015) *Tridax procumbens* Flavonoids Promote Osteoblast Differentiation and Bone Formation. *Biol Res* 48: 56. [[Crossref](#)]
- Al Mamun MA, Hosen MJ, Khatun A, Alam MM, Al Bari MA, et al. (2017) *Tridax procumbens* Flavonoids: A Prospective Bioactive Compound Increased Osteoblast Differentiation and Trabecular Bone Formation. *Biol Res* 50: 28. [[Crossref](#)]
- Al Mamun MA, Asim MMH, Sahin MAZ, Alam MM, Al Bari MA, et al. (2019) *Tridax Procumbens* Flavonoids Stimulated Synergistic Effects on BMP-2-Induced Bone Regeneration in Critical-Sized of Calvarial Defect. *J Dent Oral Health* 6: 1-8.
- Wikler MA (2006) Performance Standards for Antimicrobial Susceptibility Testing, Sixteenth Informational Supplement, M100-S16. *Clinical and Laboratory Standards Institute* 26: 3.
- Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45: 493-496. [[Crossref](#)]
- Holder IA, Boyce ST (1994) Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. *Burns* 20: 426-429. [[Crossref](#)]
- Okwu DE, Josiah C (2006) Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology* 5: 357-361.
- Akinpelu DA (2000) Antimicrobial activity of *Bryophyllum pinnatum* leaves. *Fitoterapia* 71: 193-194. [[Crossref](#)]