E-ISSN: 2378-654X

Recent Advances in Biology and Medicine

Original Research Article

Antimicrobial Activity of *Kalanchoe blossfeldiana* and *Paederia foetida* Plant Leaves' Extracts Against Some Selected Bacterial Strains

HATASO

Antimicrobial Activity of *Kalanchoe blossfeldiana* and *Paederia foetida* Plant Leaves' Extracts Against Some Selected Bacterial Strains

Arnaba S Chaity[1,2], Tamanna Nasrin[3], Khandker J Ferdouse[2,4], Md. Fazlul Haque[3], Md. Asadul Islam[2], Biswanath Sikdar[2], Md. Faruk Hasan[2]*

[1]Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh.
[2]Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi, Bangladesh.
[3]Department of Zoology, University of Rajshahi, Rajshahi, Bangladesh.
[4]Department of Agricultural Extension, Kharmarbari, Farmgate, Dhaka, Bangladesh.

***Correspondence:** faruk_geb@ru.ac.bd

Received: Apr 28, 2020; Accepted: Jul 7, 2020

Citation: Chaity AS, Nasrin T, Ferdouse KJ, Islam MA, Sikdar B, Hasan MF. Antimicrobial activity of *Kalanchoe blossfeldiana* and *Paederia foetida* plant leaves' extracts against some selected bacterial strains. Recent Adv Biol Med. 2020; *6*(3): 1121418. https://doi.org/10.18639/RABM.2020.1121418

Abstract

There is an alternative approach to control the infectious diseases caused by pathogenic bacteria, especially resistant bacteria. This study was designed to determine the antimicrobial activities of *Kalanchoe blossfeldiana* and *Paederia foetida* plants' extracts against some selected bacterial strains. *K. blossfeldiana* and *P. foetida* leaves were extracted in methanol. *In vitro* antibacterial activities were evaluated against 12 bacterial strains including, *Staphylococcus gallinarum*, *Staphylococcus sciuri*, *Streptococcus constellatus*, *Streptococcus iniae*, *Aeromonas diversa*, *Xanthomonas campestris*, *Xanthomonas axonopodis*, *Siccibacter colletis*, *Edwardsiella anguillarum*, *Aeromonas cavernicola*, *Enterobacter xiangfangensis*, and *Vibrio rotiferianus*. Antimicrobial activities were screened by disk diffusion method. In addition, minimum inhibitory concentration (MIC) was determined using broth dilution method. Data were analyzed using SPSS 16 statistical software. In antimicrobial screening, both the plant extracts showed highest inhibition (15 mm zone diameter) against *S. gallinarum* at the concentration of 20 µg/disk and 15 µg/disk, respectively. In the MIC test, both *K. blossfeldiana* and *P. foetida* leaves' extracts showed the lowest MIC value of 100 µg/ml on *V. rotiferianus* and *S. iniae*, respectively. From the above findings, it can be concluded that the extracts may be used as natural antibacterial agent for the treatment of some bacterial diseases. Further investigations on the chemical composition and possible isolation of active ingredients are warranted.

Keywords: Antimicrobial activity; Bacteria; *Kalanchoe blossfeldiana*; Leaves' extracts; *Paederia foetida.*

1. INTRODUCTION

Since the beginning of the human civilization, medicinal plants have played a vital role [1]. Over the last few centuries, people's obsession with finding an alternate approach to the current medicinal systems in curing diseases and maintaining good health has led to the increased use of medicinal herbs. Decoction of an entire plant has traditionally been used in Ayurveda medicine for the treatment of various kinds of diseases such as arthritic, spasmodic, diaphoretic, expectorant, and stomachic diseases. Decoctions are also used in the management of asthma, bowel complaints, diarrhea, diabetes, and seminal weakness. Dried fruits are also used and the extracts are applied to relieve toothache too [2]. Many important drugs and medicines of modern times have originated from plants [3]. Medicinal plants play a main role and constitute the backbone in all the traditional medicine. An essential first step is the establishment of standards of quality, security, and effectiveness to confirm the safe use of these medicines [4]. Keeping this fact in the consideration, the challenge was to establish the physiochemical values of the traditional medicinal plants.

Kalanchoe blossfeldiana is a perennial, herbaceous, bushy, and evergreen plant with shiny-textured glossy foliage, belonging to the family of *Crassulaceae*. It is an indoor plant, which has the potential to absorb toluene and ethyl-benzene to minimize the in-house air pollution [5]. The leaves of *K. blossfeldiana* contain anthocyanin, which has an antioxidant property [6]. The plant has inflorescence of varied colors due to presence of various components like 3,5-o-beta-D-diglucosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin at varied concentrations [7].

Paederia foetida is a slender, perennial herb belonging to the family of *Rubiaceae* [8]. It tastes bitter and has a foul smell. Sometimes this plant is kept as an ornamental plant and has virtue in folk medicine. It is also used as a culinary spice in some traditional cooking in North Eastern and Eastern India [9]. The plant is used in the treatment of gout, vesical

calculi, diarrhea, dysentery, piles, and inflammation of the liver and also as an emetic drug. The major classes of chemical components present in this plant are iridoid glycosides, sitosterol, stigmasterol, alkaloids, carbohydrates, protein, amino acid, and volatile oil [8].

Both *K. blossfeldiana* and *P. foetida* play important roles in folk and traditional medicine and they are used as food supplement as well. There are reports available on different aspect of these plants. As far our knowledge goes, there is no sufficient information available on the antimicrobial capability of these two important medicinal plants against tested pathogenic bacterial strains that cause some devastating diseases in animals and plants. Therefore, the aim of this study was to investigate the antibacterial activity of *K. blossfeldiana* and *P. foetida* plant leaves' extracts against some pathogenic bacteria and to determine their minimum inhibitory concentration (MIC).

2. METHOD(S)

2.1. Types of Study

This descriptive study was conducted during the period from January 2019 to November 2019, in Professor Joarder DNA and Chromosome Research Lab, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi, Bangladesh.

2.2. Plant Materials Collection

Fresh leaves of *K. blossfeldiana* and *P. foetida* were collected from the University of Rajshahi, Rajshahi, Bangladesh. The leaves were cleaned with sterile distilled water.

2.3. Collection of Bacterial Stains and Culture

Twelve pathogenic bacteria, previously isolated and identified, were collected from Microbiology laboratory, Department of Genetic Engineering and Biotechnology. Four of them are Gram-positive, which are *Streptococcus constellatus*, *Staphylococcus gallinarum*, *Staphylococcus sciuri*, and *Staphylococcus iniae* and eight of them are Gram-negative, which are *Aeromonas diversa*, *Xanthomonas campestris*, *Xanthomonas axonopodis, Siccibacter colletis, Edwardsiella anguillarum*, *Aeromonas cavernicola*, *Enterobacter xiangfangensis*, and *Vibrio rotiferianus*. All the bacterial strains were cultured in Lauria–Bertani (LB) broth and LB agar media for next works.

2.4. Extraction and Fractionation

After cleaning the impurities from the leaves, the plant material was then air dried in room temperature. After 7 days, the dried plant material was ground in a blender to form a fine powder. This powder was used for the preparation of methanol extracts by sequential extraction [10]. Dried powder of plants (100 g of each plant) was extracted by methanol (250 ml/100 g powder) using conical flask, by shaking and stirring for 14 days. To obtain the large quantity of extracts, the content was pressed through the marking cloth and the whole mixture was then filtered using Whatman no. 1 filter paper after that the remaining filtrate was dehydrated *in vacuo* to afford a blackish mass. Then the remaining output extracts and fraction were collected in vials and conserved in a refrigerator at 4°C carefully.

2.5. Disk Preparation

The Whatman no. 1 filter paper was punched with a punching machine and was made into a disk of size 6 mm. The disk filter paper was taken into the test tubes and sterilized in an autoclave for 15 minutes at 15 psi and 121°C temperature.

2.6. Culture Medium Preparation

In this study, LB agar medium was used for antibacterial screening. For the test, 2.8 g of the nutrient agar media was taken into a 500-ml autoclave conical flask. The media was properly dissolved in the distilled water and then sterilized in an autoclave for 15 minutes at 121°C. After autoclaving, the media was cooled for some time and then poured into the autoclaved petri dishes in the laminar airflow cabinet.

2.7. Inoculum Preparation

For inoculum preparation, 1 ml of distilled water was taken into a screw-capped tube and the pure colony of freshly cultured bacteria was added into the tube and vortexes. The OD was measured with the colorimeter and the microbial population was confirmed to be within the tube. This suspension was used as inoculums.

2.8. Antibacterial Activity of Plants' Extracts

The antibacterial activities of the plants' extracts were determined by disk diffusion method [10]. For performing the test, 250 µl of fresh broth culture containing the isolated bacteria was poured evenly on to a nutrient agar plate and spread with a sterilized glass spreader. Disks were soaked on the plants' extracts with a selected amount/disk. The amount of 15 µg/disk of each plant

extracts were taken with the help of micropipette. Standard kanamycin was used as positive control and methanol as negative control. All strains used in the study were inoculated to nutrient agar and incubated at 37°C for 14 hours and were allowed to grow until they reached 108-109 cfu/ml. Finally diameters of zone of inhibition formed due to using plant extract were measured.

2.9. Determination of Minimum Inhibitory Concentration

The rate of MIC values was determined using different concentrations of 100, 120, 130, 150, 160, and 200 µg/ml of plant extract suspensions against the tested bacteria. MIC was measured with the colorimeter and microbial population was confirmed according to McFarland turbidity method as mentioned earlier by Cockerill *et al*. [11] in which various types of concentrations of methanol extracts dilutions were prepared. The lowest concentration of the extract required to inhibit the growth of the organism *in vitro* is the MIC value. In this study, it was determined following the dilution technique. Standard kanamycin was used as positive control and methanol as negative control for comparison of the tested plant extracts' MIC values. All the treated tubes were incubated at 37°C for 1 day and the MIC was recorded [12].

2.10. Statistical Analysis

The statistical analysis was performed by using Microsoft Excel software (version 2013) and SPSS 16 to find out significant differences in the antibacterial effects. Three replicates were used for measuring the averages of the results. Results were expressed as mean ± SEM, and statistical significance was accepted at $p < 0.05$.

3. RESULTS

3.1. Antimicrobial Study

This study showed that the extract of methanol at a concentration of 15 µg/disk produced zones of inhibition, and among the 12 bacterial strains, *Staphylococcus constellatus* had the highest zone of inhibition at 15 mm and no zone of inhibition noted in *Aeromonas diversa.* The results of antimicrobial activities are presented in Figures 1 and 2.

This study showed that the extract of methanol at a concentration of 15 µg/disk produced zones of inhibition, and among the 12 bacterial strains, *Staphylococcus sciuri* had the highest zone of inhibition at 15 mm and no zone of inhibition noted in *Aeromonas diversa, Xanthomonas campestris*, *Streptococcus constellatus, Staphylococcus gallinarum, Aeromonas diversa*, and *Siccibacter colletis*; standard kanamycin (5 µg/disk) showed a 7-18-mm zone of inhibition. The results of antimicrobial activities are presented in Figures 1 and 3.

The control standard kanamycin (5 µg/disk) was used as positive control, which showed a zone of inhibition of 7–18 mm against the tested bacteria (data not showed). Methanol was used as negative control, which showed no zone of inhibition against the tested bacteria (data not showed).

3.2. Determination of MIC Values

In MIC value test, *K. blossfeldiana* and *P. foetida* leaves' extracts were used against 12 bacterial strains at different concentrations. The MIC values ranged from 100 to 200 µg/ml against the tested Gram-negative and Gram-positive bacteria. Methanol was

Figure 1: Antibacterial activities of *K. blossfeldiana* and *P. foetida* leaves' extracts against the tested bacteria.

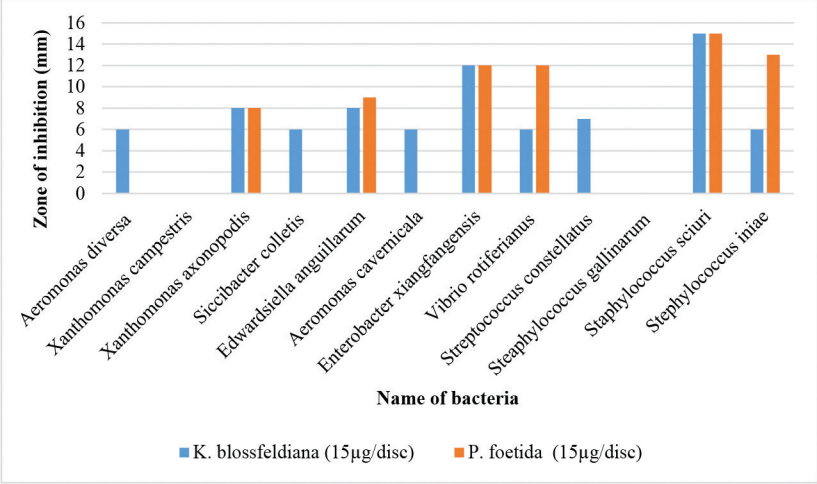


Figure 2: Antibacterial activities of *K. blossfeldiana* leaves' extract against the tested bacteria. (A) *Aeromonas diversa*, (B) *Xanthomonas axonopodis*, (C) *Siccibacter colletis*, (D) *Aeromonas cavernicola*, (E) *Enterobacter xiangfangensis*, (F) *Vibrio notiferianus*, (G) *Steptococcus constellatus*, and (H) *Staphylococcus sciuri*; the concentration of 15 µg/disk in a 60-mm petridish.

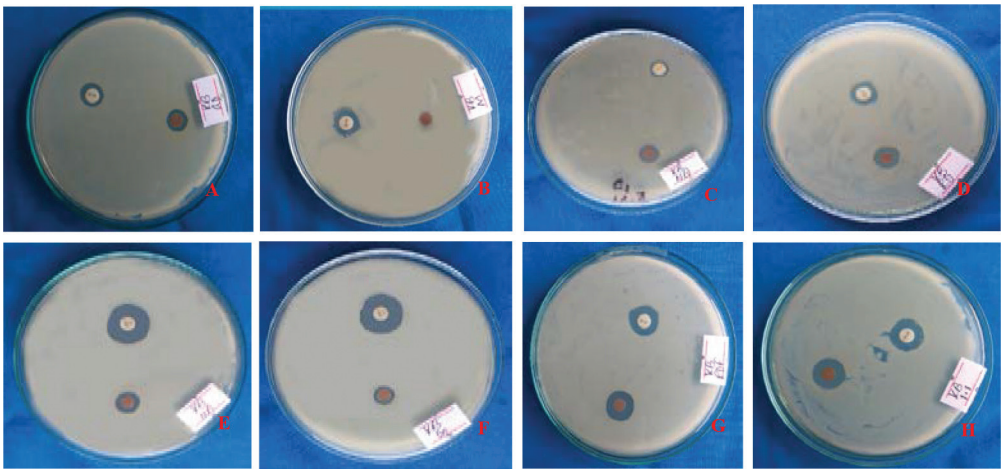
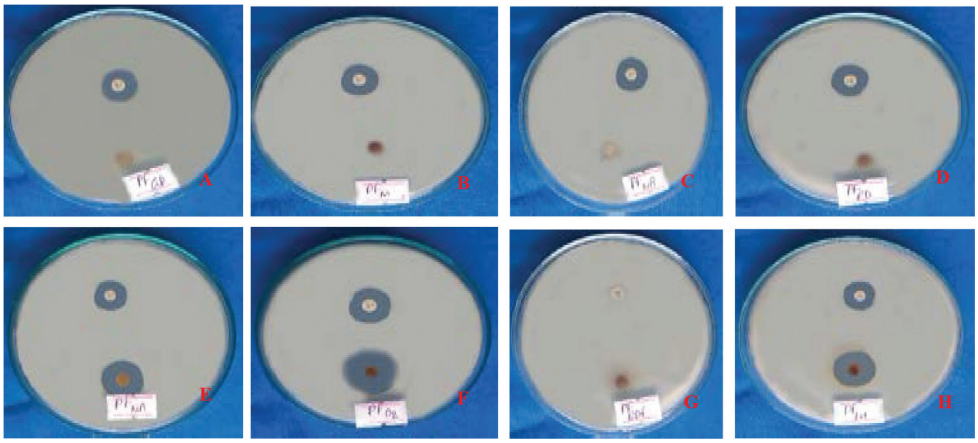


Figure 3: Antibacterial activities of *P. foetida* leaves' extract against the tested bacteria. (A) *Aeromonas diversa*, (B) *Xanthomonas axonopodis*, (C) *Siccibacter colletis*, (D) *Aeromonas cavernicola*, (E) *Enterobacter xiangfangensis*, (F) *Vibrio notiferianus*, (G) *Streptococcus constellatus*, and (H) *Staphylococcus sciuri*; the concentration of 15 µg/disk in a 60-mm petridish.



used as negative controls, which showed no inhibition against all the organisms (data not showed). The standard antibiotic kanamycin was used as positive control, which showed MIC value varying from 10 to 30 µg/ml against the tested bacteria (data not given). The results are presented in Figures 4-6.

4. DISCUSSION

In this study, *K. blossfeldiana* and *P. foetida* extracts exhibited growth inhibition against eight Gram-negative species and four Gram-positive bacteria. Both the plants, *K. blossfeldiana* and *P. foetida*, showed significant inhibitory effect against all the tested bacteria, except *Xanthomonas campestris* and *Staphylococcus gallinarum*. The highest inhibition zone of Gram-negative bacteria was 12 mm in diameter—it was found against *E. xiangfangensis* and this indicates that it is weaker than other bacteria. Similarly the lowest inhibition zone of Gram-negative bacteria was 5 mm found for *A. diversa*, *S. colletis*, and *A. cavernicola*, which indicates that these are stronger bacteria. The plant extract showed no inhibition zone against *X. campestris* and *S. gallinarum*. At the same time, the highest inhibition zone of Gram-positive bacteria is 15 mm in diameter found against *S. sciuri* and the lowest inhibition

Figure 4: MIC values of *K. blossfeldiana* and *P. foetida* leaves' extracts against the tested bacteria.

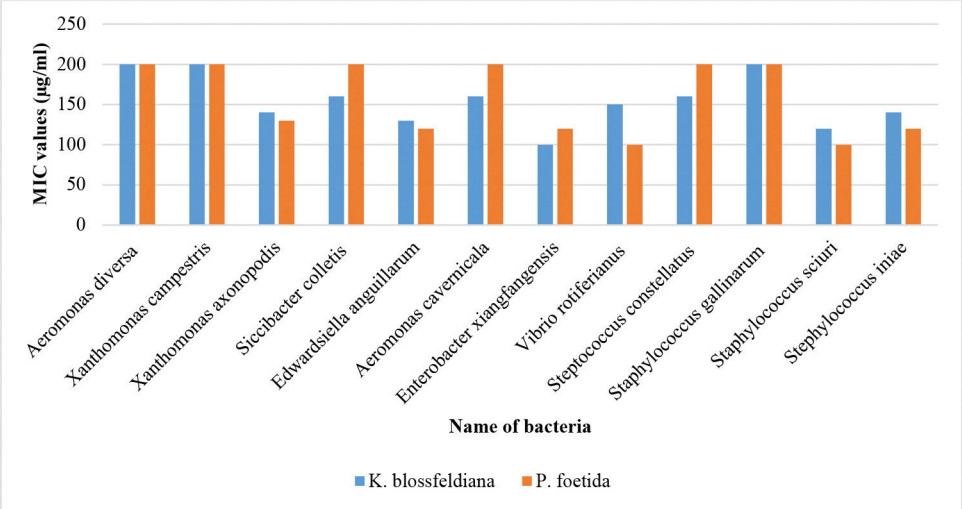
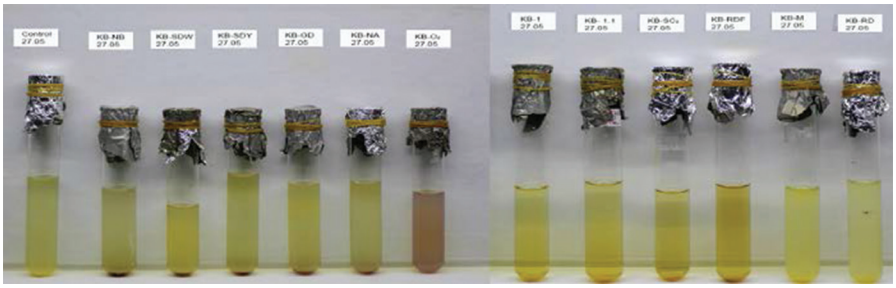
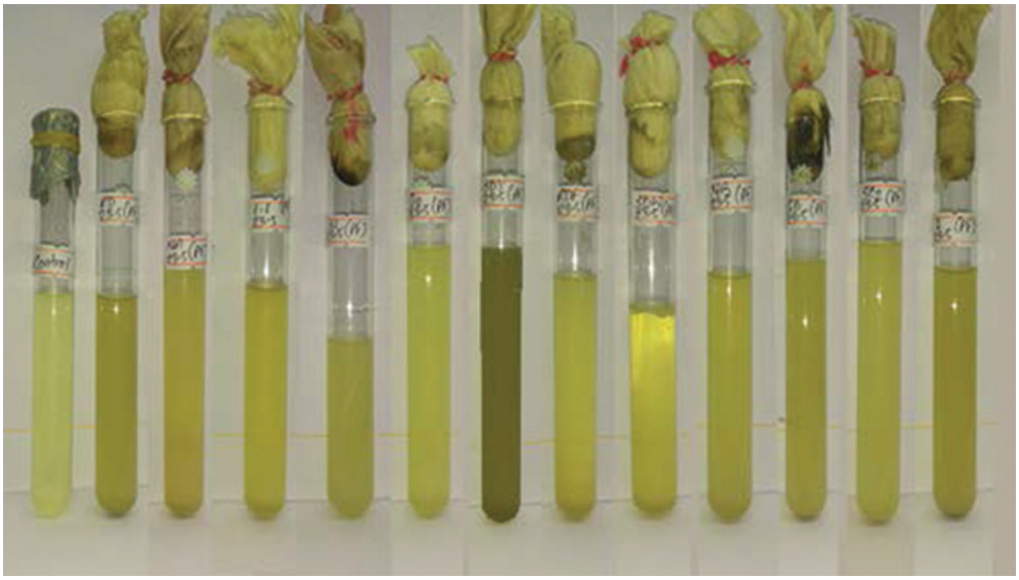


Figure 5: MIC values of *K. blossfeldiana* leaves' extracts against *Streptococcus constellatus*, *Staphylococcus gallinarum*, *Staphylococcus sciuri*, *Streptococcus iniae*, *Aeromonas diversa*, *Xanthomonas campestris*, *Xanthomonas axonopodis*, *Siccibacter colletis*, *Edwardsiella anguillarum*, *Aeromonas cavernicola*, *Enterobacter xiangfangensis*, and *Vibrio rotiferianus* at the concentrations of 100, 120, 130, 150, 160, and 200 µg/ml.



zone is 4 mm in diameter found against *S. iniae*. Our present findings support the previous work, where *Kalanchoe spp*. extracts tested against bacteria exhibited growth inhibitory effects more readily against Gram-positive pathogens [13]. *K. fedtschenkoi* extract exhibited growth inhibition against two Gram-negative sp. *A. baumannii* (CDC-33) and *P. aeruginosa* (AH-71) as well as Gram-positive *S. aureus* [14]. Extract in other studies with *S. aureus* have always shown growth inhibition with the exception of the poor performance of a hexane fraction tested [15]. The dichloromethane extract of *Kalanchoe pinnata* plant leaves exhibited maximum antimicrobial activity against *E. coli* followed by *Coccinia galabrata* and *C. parapsilosis* plants [16]. In this study, the reducing activity increased with respect to the concentration of different organic extract (dichloromethane extract, ethyl acetate, and methanol) of *K. pinnata*. Methanol extract of *K. blossfeldiana*, which at a concentration of 200 µg/ml, used to control the pathogenic bacteria but gives the best result in *S. sciuri*. Uddin *et al.* [17] reported that crude extract of *P. foetida* extracted using ethanol had antimicrobial activity against Gram-negative bacteria including *S. flexneri* and *E. coli* with the zone of inhibition ranging from 17 to 27 mm at a concentration of 25 to 75 µg/ml and there was no inhibitory effect based on the MIC value (at low concentration, <25 µg/ml) against *S. flexneri* and *E. coli*. According to Upadhyaya [18], the ethanol extract of *P. foetida* exhibited no inhibitory effect against *Proteus vulgaris, E. coli*, and *Pseudomonas aeruginosa* in the MIC test. The methanol extract of *P. foetida* showed significant antibacterial activity using MIC value determination method. The experiments also revealed that n-hexane extract possessed a very less antifungal activity for *Candida albicans* and *Saccharomyces cerevisiae* [19]. Methanol extracts of *P. foetida* leaf tissue were most effective in inhibiting *in vitro* growth of the eight MDR enteropathogens [20]. Similarly methanol extract of *P. foetida*, which at a concentration of 200 µg/ml, used to control the pathogenic bacteria but gives the best result in *Streptococcus iniae* bacteria. Similar results were reported by Chaity *et al.* [21] for *Rumex vesicarius* leaves' extract and by Shahen *et al.* [22] for *Calendula officinalis* in petroleum ether against *K. pneumoniae* and *E. coli*, which support our present findings.

Figure 6: MIC values of *P. foetida* plant leaves' extracts against *Streptococcus constellatus*, *Staphylococcus gallinarum*, *Staphylococcus sciuri*, *Streptococcus iniae*, *Aeromonas diversa*, *Xanthomonas campestris*, *Xanthomonas axonopodis*, *Siccibacter colletis*, *Edwardsiella anguillarum*, *Aeromonas cavernicola*, *Enterobacter xiangfangensis*, and *Vibrio rotiferianus* at the concentrations of 100, 120, 130, 150, 160, and 200 µg/ml.



According to Kuete [23], activity of crude extracts is considered to be significant if MIC values are below 100 µg/ml, moderate when 100<MIC<625 µg/ml, or low when MIC > 625 µg/ml.

In this study, we used methanol extract only. So, for authentic research, other solvent should be used. Also, only 12 bacterial strains are not enough for detection of antimicrobial activities of the tested plant extracts. Moreover, antifungal activities also should be determined. The present research revealed that both the plant leaves' extracts *K. blossfeldiana* and *P. foetida* have significant antibacterial activities against the tested bacterial strains.

Therefore, our results revealed the importance of *K. blossfeldiana* and *P. foetida* plant extracts to control resistant bacteria, which are becoming a threat to human health as well as plants. Furthermore, in a few cases, both plants extracts were active against antibiotic resistant bacteria under medium concentration, thus minimizing the possible toxic effects.

5. CONCLUSION

All over the world, in our daily lives, for years we have been utilizing the beneficial health effects of the extracts from many types of plants to treat various diseases. In this study, the extracts of plants' leaves displayed a variable degree of antimicrobial activity on different pathogenic bacteria. This study exposed that the methanol extract from leaves of *K. blossfeldiana* and *P. foetida* were exhibiting antibacterial activity, which might be helpful in inhibiting the resistant bacterial infections. The plants' extracts can be used as a substitute agent of medicine because of their bioactive compounds. However, further studies are necessary to find the mechanism of the extracts' antibacterial efficacy and to analyze the active compounds responsible for this biological activity. The purpose of this study was to examine the inhibitory effects of *K. blossfeldiana* and *P. foetida* leaves' extracts on some bacteria that cause poisoning in humans and plants and are thus considered harmful. So, further microbiological investigation was confined only on methanol fraction and also more investigation is necessary to confirm the bioactive principles of the valuable medicinal plants in Bangladesh.

Acknowledgment

The authors thank the Institute of Biological Sciences, Professor Joarder DNA & Chromosome Research Lab and Department of Zoology, University of Rajshahi, Rajshahi, Bangladesh, for providing laboratory facilities to carry out the research work.

Authors' Contributions

This work was carried out with the collaboration of all authors. ASC and MFH designed the experiments, developed the methodology, and prepared the manuscript. ASC, TN, and MFH collected the data and carried out analysis. ASC, MFH, AI, BS, and MFH assisted with manuscript preparation. All authors read and approved the final manuscript.

Conflict of Interest

Authors declare that no competing interest exists.

References

1. Nostro A, Germano MP, D'angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol. 2000; 30(5):379-84.
2. Panda AK. Medicinal plants of Sikkim in Ayurvedic practice. Fourth Edition. Tadong, Gangtok, Sikkim (1987), 1: 5 p.
3. Thomas S, Patil DA, Patil A, Chandra N. Pharmacognostic evaluation and physicochemical analysis of *Averrhoacarambola* L. fruit. J Herb Med Toxicol. 2008; 2(2):51-4.
4. Kumar V, Pankajkumar YS, Singh UP, Bhat HR, Zaman MK. Pharmacognostical and phytochemical study on the leaves of *Paederia foetida* linn. Int J Pharm Tech Res. 2009; 1(3):918-20.
5. Sriprapat W, Suksabye P, Areephak S, Klantup P, Waraha A, Sawattan A, *et al.* Uptake of toluene and ethylbenzene by plants: removal of volatile indoor air contaminants. Ecotox Environ Safe. 2014; 102:147-51.
6. Neyland M, Ng YL, Thimann VK. Formation of anthocyanin in leaves of *Kalanchoe blossfeldiana* - a photoperiodic response. Plant Physiol. 1963; 38(4):447.
7. Nielsen AH, Olsen C, Møller BL. Flavonoids in flowers of 16 *Kalanchoe blossfeldiana* varieties. Phytochemistry. 2005; 66(24):2829-35.
8. Blatter E, Caius JF. Indian medicinal plants. Volume II. CSIR, New Delhi (1981), 1297-9 pp.
9. Chanda SD, Lokesh T, Singh RK, Sayeed KA. Gastroprotective mechanism of *Paederia foetida* Linn. (Rubiaceae) – a popular edible plant used by the tribal community of North-East India. BMC Complement Altern Med. 2015; 15(1):304.
10. Hasan MF, Sikdar B. Screening of antimicrobial, cytotoxic and pesticidal activities of *Coccinia grandis* (L.) Voigt. J Microbiol Biotech Food Sci. 2016; 5(6):584-8.
11. Cockerill FR, Bradford P, Eliopoulos GM, Hindler JA, Jenkins SG, Lewis J, *et al*. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard. Ninth Edition. CLSI, Wayne, PA (2012), 12 p.
12. Otang WM, Grierson DS, Ndip RN, Hoffm O. Antifungal activity of *Arctotisarctotoides* (L) and Gasteria bicolor H. against opportunistic fungi associated with human immunodeficiency virus/acquired immunodeficiency syndrome. Pharmacogn Mag. 2012; 8:135-40.
13. Akinsulire OR, Aibin I, Adenipekun T, Adelowotan T, Odugbemi T. *In vitro* antimicrobial activity of crude extracts from plants *Bryophyllum pinnatum* and *Kalanchoe crenata*. Afr J Tradit Complement Altern Med. 2007; 4:338-44.
14. Richwagen NJ, Lyles T, Dale BL, Quave CL. Antibacterial activity of *Kalanchoe mortagei* and *K. fedtschenkoi* against ESKAPE pathogens. Front Pharmacol. 2019; 10:67.
15. Haier R, Karama JS, Leyba L, Jung RE. MRI assessment of cortical thickness and functional activity changes in adolescent girls following three months of practice on a visual-spatial task. BMC Res Notes. 2009; 2(1):174.
16. Akinpelu DA. Antimicrobial activity of *Bryophyllum pinnatum* leaves. Fitoterapia. 2000; 71(2):193-4.
17. Uddin BT, Nahar T, Khalil MI, Hossain S. *In vitro* antibacterial activity of the ethanol extract of *Paederia foetida* L. (Rubiaceae) leaves. J Life Sci. 2007; 19:141-3.
18. Upadhyaya S. Screening of phytochemicals, nutritional status, antioxidant and antimicrobial activity of *Paederia foetida* Linn. From different localities of Assam, India. J Pharmacol Res. 2013; 7:139-41.
19. Morshed H, Islam MS, Parvin S, Ahmed MU, Islam M, Mostofa AG, *et al.* Antimicrobial and cytotoxic activity of the methanol extract of *Paederia foetida* Linn. (Rubiaceae). J Appl Pharm Sci. 2012; 2(1):77-80.
20. Rath S, Padhy RN. Antibacterial efficacy of five medicinal plants against multidrug-resistant enteropathogenic bacteria infecting under-5 hospitalized children. J Integr Med. 2015; 13(1):45-57.
21. Chaity AS, Islam MA, Nasrin T, Sarker SR, Dutta AK, Sikdar B, *et al.* Screening of *in vitro* antibacterial activity of *Rumex vesicarius* (L.) leaves extract against twelve pathogenic bacteria. J Adv Microbiol. 2019; 19(4):1-6.
22. Shahen MZS, Mahmud SN, Sohana MH, Rony MA, Imran M, Al Maruf MAA, *et al*. Effect of antibiotic susceptibility and inhibitory activity for the control of growth and survival of microorganisms of extracts of *Calendula officinalis*. Eur J Med Health Sci. 2019; 1(3):1-9.
23. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med. 2010; 76:1479-791.