Antimicrobial activity of Kalanchoe blossfeldiana and Paederia foetida leaves extracts against some selected bacterial strains

Accepted 4th June, 2020

ABSTRACT

There is an alternative approach to control the infectious diseases caused by pathogenic bacteria especially resistant bacteria. The present study was design to determine the antimicrobial activities of Kalanchoe blossfeldiana and Paederia foetida plants extracts against some selected bacterial strains as well as their bacterial potency. K. blossfeldiana and P. foetida leaves were extracted in methanol. In vitro antibacterial activities were evaluated against twelve bacterial strains viz., Streptococcus constellatus, Staphylococcus gallinarum, Staphylococcus sciuri, Streptococcus iniae, Aeromonas diversa, Xanthomonas campestris, Xanthomonas axonopodis, Siccibacter colletis, Edwardsiella anguillarum, Aeromonas cavernicula, Enterobacter xiangfangenensis and Vibrio rotiferianus. Antimicrobial activities were screened using disc diffusion method. In addition, minimum inhibitory concentration (MIC) was determined using serial dilution method. In antimicrobial screening, both the plant extracts showed highest 15 mm zone of inhibition against Staphylococcus gallinarum at the concentration of 20 and 15 μg/disc, 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 Strept. iniae, respectively. From the above findings, it can be concluded that the present research information may be helpful in the use of natural antibacterial agent for the treatment of some bacterial diseases.

Key words: Kalanchoe blossfeldiana, Paederia foetida, leaves extracts, bacteria, antimicrobial activity.

INTRODUCTION

Medicinal plants have been playing a leading role since the beginning of the human civilization (Nostro et al., 2000). Obsession on the present medicinal system leads people to another approach to recover and maintain good health, which increased extremely by using medicinal herb over the last centuries. The decoction of the entire plant is traditionally used in Ayurveda medicine for the treatment of various kinds of diseases such as antiarthritic, antispasmodic, diaphoretic, expectorant and stomachic. It is also used for the management of asthma, bowel complaints, diarrhea, diabetes, and seminal weakness. The dried fruits are also used and the extract is applied for toothache as well (Panda, 1987). Many important drugs and treatment medicines of modern times are from plant origin (Thomas et al., 2008). Medicinal plants play major role and constitute the backbone of all traditional medicine. An essential first step is the establishment of standards of quality, security and effectiveness to confirm the safe use of these medicines (Kumar et al., 2009). Keeping this fact in consideration, the challenges were made to establish 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 ethylbenzene to minimize the in-house air pollution (Sriprapa...
et al., 2014). The leaves of *K. blossfeldiana* contain anthocyanin, which has antioxidant activity (Neyland et al., 1963). The plant have inflorescence of varied colors due to presence of various components such as 3,5- o-beta-D-diglucosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin at varied concentrations (Nielsen et al, 2005).

*Paederia foetida* is a slender, perennial herb belonging to the family of Rubiaceae (Blatter et al., 1981). It tastes bitter with having foul smell. Sometimes this plant is planted as an ornamental, and has virtue in folk medicine. It is also used as a culinary spice in some traditional cooking in North Eastern and Eastern India (Chanda et al., 2015). The plant is used in gout, vesical calculi, diarrhoea, dysentery, piles, and inflammation of the liver and emetic treatment. The major classes of chemical component present in this plant are iridoid glycosides, stigmasterol, sitosterol, alkaloids, carbohydrates, protein, amino acid and volatile oil (Blatter et al., 1981).

Both *K. blossfeldiana* and *P. foetida* plants are very important in folk and traditional medicine as well supplement of food. There are some reports in different aspect of these plants. To the best of our knowledge, there is no sufficient report on antimicrobial capability of these two important medicinal plants against the tested pathogenic bacterial strains which cause some devastating diseases for animals and plants. Therefore, the aim of the present study was to investigate the antibacterial screening of *K. blossfeldiana* and *P. foetida* plant leaves extracts against some pathogenic bacteria and determine their minimum inhibitory concentration.

**MATERIALS AND METHODS**

**Plant materials collection**

Healthy, disease free, mature *K. blossfeldiana* and *P. foetida* plants were collected from the University of Rajshahi Campus, Rajshahi-6205, Bangladesh and identified by the Department of Botany, University of Rajshahi, Bangladesh. The leaves were collected from the plants. The surfaces of the leaves were cleaned with sterile distilled water and were used as plant materials.

**Collection of bacteria stains and culture**

Twelve pathogenic bacteria were collected from Microbiology laboratory, Department of Genetic Engineering and Biotechnology, Rajshahi University, Rajshahi 6205, Bangladesh which were previously isolated and identified. Four of them were gram positive bacteria *Streptococcus constellatus*, *Staphylococcus gallinarum*, *Staphylococcus sciuri*, *Streptococcus iniae* and eight were gram negative bacteria *Aeromonas diversa*, *Xanthomonas campestris*, *Xanthomonas axonopodis*, *Siccibacter colletis*, *Edwardsiella anguillarum*, *Aeromonas cavernical*, *Enterobacter xiangfangensis* and *Vibrio rotiferianus*. All the bacterial strains were cultured in LB broth and agar media for further analyses.

**Extraction and fractionation**

After cleaning the waste materials of the leaf, the plant material was air dried at room temperature. After 7 days, the dried plant was grinded to fine powder using blender machine. The powder form was used for the preparation of methanol extracts by sequential extraction. Dried powder of plants (100gm of each plant) was extracted by methanol (250 ml/100gm powder) using conical flask, through 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. Thereafter, the remaining filtrates were 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.

**Disk preparation**

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

**Culture medium preparation**

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

**Inoculum preparation**

For inoculum preparation, 1 ml of distilled water was taken into the 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 microbial population was confirmed to be within the tube. This suspension was used as inoculums.

**Antibacterial activity of plants extracts**

The antibacterial activities of the plants extracts were
determined using disk diffusion method (Hasan and Sikdar, 2016). To perform the test, 250 μl of fresh broth culture containing isolated bacteria was pour evenly on a nutrient agar plate and spread with a sterilized glass spreader. Selected amount of discs were soaked in the plants extracts solution. The amount of 15 μg/disc 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 h and were allowed to grow until they reach 108-109 cfu/ml. Finally, diameters of zone of inhibition were formed due to the use of plant extract and then measured by mm scale.

**Determination of minimum inhibitory concentration (MIC)**

The rate of MIC values were determined using serial dilutions of 100, 120, 130, 150, 160, and 200 μg/ml in methanol solvent against the tested bacteria in agar well diffusion method as mentioned earlier by Saikot et al. (2012) in which various types of concentrations of methanol extracts serial dilutions were prepared. The lowest concentration of the extract required to inhibit the growth of the organism in vitro is MIC. In the present study, it was determined following the serial dilution technique. Standard Kanamycin was used as positive control and methanol as negative control for comparison of the tested plants extracts MIC values. All the treated tubes were incubated for 48 h at 37°C.

**Statistical analysis**

The statistical analysis was performed using Microsoft Excel software, version 2013 plus professionals to determine significant differences in antibacterial effects. Three replicates were used for measuring the averages of the results.

**RESULTS**

**Antimicrobial study**

The study showed that the extract of methanol at a concentration of 15 g/disc had zone of inhibition produced in case of 12 bacterial strains, where the highest zone of inhibition of 15 mm was observed in *Staph. sciuri* and no zone of inhibition was found in *A. diversa, X. campestris, Strept. constellatus, Staph. gallinarum*, and *S. colletis*, standard Kanamycin (5 μg/disc) showed 7-18 mm zone of inhibition. The results of the antimicrobial activities are shown in Figures 1 and 3.

The control standard Kanamycin (5 μg/disc) was used as positive control which showed zone of inhibition of 7-18 mm against the tested bacteria (data not shown). Methanol was used as negative control which showed no zone of inhibition against the tested bacteria (data not shown).

**Determination of MIC values**

In MIC values test, *K. blossfeldiana* and *P. foetida* leaves extracts were used against twelve bacterial strains at different concentrations. The MIC values ranged from 100 to 200 μg/ml respectively against the tested gram negative and positive bacteria. Methanol was used as negative controls which showed no inhibition against all the organisms (data not shown). The standard antibiotic kanamycin was used as positive control which showed MIC value that varied from 10 to 30 μg/ml against the tested bacteria (data not given). The results are shown in Figures 4 to 6.

**DISCUSSION**

In the present study, *K. blossfeldiana* and *P. foetida* extracts exhibited growth inhibition against eight Gram-negative species and four gram positive bacteria. Both *K. blossfeldiana* and *P. foetida* plants showed significant inhibitory effect against all the tested bacteria, except *X. campestris* and *Staphy. gallinarum*. The highest inhibition zone of gram negative bacteria was 12 mm in diameter found against *E. xiangfangensis*, indicating that it is weak as compared with other bacteria. Similarly, the lowest inhibition zone of gram negative bacteria was 5 mm found in *A. diversa*, *S. colletis* and *A. cavernicala*, indicating that they are strong 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 was 15 mm in diameter found against *S. sciuri* and lowest inhibition zone was 4 mm in diameter found against *S. iniae*. The findings of the present study are in line with previous report, where *Kalanchoe* spp. extracts tested against bacteria exhibited growth-inhibitory effects more readily against gram-positive pathogens (Akinsulire et al., 2007). *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 *Staph. aureas* (Richwagen et al., 2019). Extract in other studies with *Staph. aureas* have always shown growth inhibition with the exception of the poor performance of a hexane fraction
Figure 1: Antibacterial activities of *K. blossfeldiana* and *P. foetida* leaves extracts against the tested bacteria.

![Figure 1](image1.png)

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 rotiferianus*, (G) *Streptococcus constellatus*, (H) *Staphylococcus sciuri* the concentration of 15 g/disc.

![Figure 2](image2.png)

tested (Haier et al., 2009). The dichloromethane extract of *Kalanchoe pinnata* plant leaves exhibited maximum antimicrobial activity against *E. coli*, followed by *Coccinia galabrata* and *C. parapsilosis* plants (Akinpelu, 2000). In the present 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 was 200
Figure 3: Antibacterial activities of *P. foetida* leaves extract against the tested bacteria; (A) *Aeromonas diversa*, (B) *Xanthomonas axonopodis*, (C) *Siccibacter colletis*, (D) *Aeromonas cavernica* (E) *Enterobacter xiangfangensis*, (F) *Vibrio rotiferianus*, (G) *Streptococcus constellatus*, (H) *Staphylococcus sciuri* the concentration of 15 g/disc.

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

μg/ml concentration, was used to control the pathogenic bacteria but gave the best result in *S. sciuri*. Uddin et al. (2007) 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 mg/ml and there was no inhibitory effect based on the MIC value (at low concentration, <25 mg/ml) against *S. flexneri* and *E. coli*. According to Upadhyaya (2013), the ethanol extract of *P. foetida* exhibited no inhibitory effect against *Proteus vulgaris, E. coli* and *Pseudomonas auruginosa* at MIC test. The methanol extract of *P. foetida* showed significant antibacterial activity using MIC value determination method. The experiments also showed that n-hexane extract possess a very less antifungal activity on *Candida*
albicans and Sacharomyces cerevacae (Morshed et al., 2012). Methanol extracts of P. foetida leaf tissue were most effective in inhibiting in vitro growth of the 8 MDR enteropathogens (Rath and Padhy, 2015). Similarly methanol extract of P. foetida which was 200μg/ml concentration was used to control the pathogenic bacteria but gave the best result in Strept. iniae bacteria. Similar results were reported by Chaity et al. (2019) for Rumex vesicarius leaves extract and Shahen et al. (2019) for Calendul officinalis in petroleum ether against K. pneumoniae and E. coli. The present research showed that the leaves extracts of both plants K. blossfeldiana and P. foetida have significant antimicrobial activities against the tested bacterial strains.

CONCLUSIONS

The extracts of many types of plants have beneficial health effects, as they have been used for years in daily life to treat diseases all over the world. In the present study, the extracts of plants leaves showed a variable degree of antimicrobial activity on different pathogenic bacteria. This study demonstrated that the methanol extract of leaves of K. blossfeldiana and P. foetida exhibited antibacterial activity, which might be helpful in inhibiting the resistant bacterial infections. The plants extracts can be used as substitute antimicrobial agent in medicine because of their bioactive compound. However, further studies are necessary to determine the mechanism of extract of antibacterial efficacy and to analyze the active compounds responsible for this biological activity. The purpose was to examine the inhibitory effects of K. blossfeldiana and P. foetida leaves extracts, some bacteria causing poisoning and are harmful for humans and plants. The microbiological investigation was confined only on methanol fraction and as such, further investigation is necessary to confirm the bioactive principles of the valuable medicinal plants in Bangladesh.

ACKNOWLEDGEMENT

The authors would like to thank the Chairman, Department
of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh for providing the bacterial strains to carry the research work.

REFERENCES


Cite this article as:

Submit your manuscript at
http://www.academiapublishing.org/journals/jbs