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Comparative study of antibacterial activity and phytochemical analysis of stem, root and leaf extracts of *Paederia foetida* L. against phytopathogenic bacteria

Piyarat Namsena[a,*], Juthaphak Maneetap[b], Duangkamon Phonpheng[b], Walita Ngamsane[b]

[a] *Department of Biotechnology, Faculty of Science and Technology, Rajabhat Maha Sarakham University, Mahasarakham 4400, Thailand*

[b] *Department of Biology, Faculty of Science and Technology, Rajabhat Maha Sarakham University, Mahasarakham 4400, Thailand*

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Abstract

Paederia foetida L. (Skunk vine) is an indigenous weedy plant which is also known to have antimicrobial activity and to possess a broad spectrum of uses for medicinal purposes and as a food ingredient, but its beneficial effects for agriculture are still unknown. The objectives of this study were to determine the anti-phytopathogenic bacterial activity and phytochemical composition of crude extracts from different parts of *P. foetida* using 95% ethanol as a solvent and screening of crude extracts for antibacterial activity using the agar disc diffusion method. The results showed that the crude extract of roots mixed with stems and leaves of *P. foetida* (1:1:1 weight per weight) at a concentration of 100 mg/mL had highly effective antibacterial activity with inhibition zone diameters of 10.00 mm, 7.00 mm and 10.33 mm for *Xanthomonas campestris*, *Erwinia carotovora* and *Ralstonia solanacearum*, respectively. The minimal inhibitory concentrations of *P. foetida* crude extract for inhibition of *X. campestris* and *R. solanacearum* were ≥ 6.25 mg/mL and ≥ 3.15 mg/mL for *E. carotovora* and the minimal bactericidal concentrations of *X. campestris*, *E. carotovora* and *R. solanacearum* were 25 mg/mL, 6.25 mg/mL and 12.5 mg/mL, respectively. The results of phytochemical analysis of the *P. foetida* crude extracts showed that ferulic acid and luteolin were the major phytochemical components. These findings have provided the first report suggesting the effectiveness of *P. foetida* extract against phytopathogenic bacteria and revealed that the extract could be used as an alternative source of antibacterial agents for the protection of plants or crops against bacterial infection.

Introduction

Plant disease caused by phytopathogenic bacteria is one of the most important diseases affecting agricultural production worldwide as under favorable environmental conditions for the pathogen, the disease can spread very rapidly and cause severe crop losses (Stephan et al., 2005). The use of chemicals is the most common choice for management of plant disease from bacteria, but this also causes the development of bacterial resistance to these agents (Bussaman et al., 2012). And inappropriate use of chemicals to manage plant disease is not considered to be the long-term solution because this can increase the investment expenses, the risk of having high

* Corresponding authors.
E-mail address: namsenap1409@gmail.com (P. Namsena)

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levels of toxic residues and also raises concerns regarding human health and environmental settings (Latha et al., 2009). Therefore, the development of alternative agents that can replace chemicals is very desirable. The utilization of natural products including microorganisms and plant extracts is an appropriate alternative for plant disease control, especially as plant extracts have been shown to be effective against many plant pathogens and considered to be safe for consumers and environments. For example, there are reports of plant species possessing natural substances that are toxic to a variety of plant pathogenic bacteria (Fawcett and Spencer, 1970). *Paederia fotida* L., a member of the Rubiacae is an extensive climber that is glabrous or puberulous. It is known as Chinese Flower in English and is native to both temperate and tropical Asia, from India to Japan and South East Asia (Soni et al., 2013. It has a bitter taste and a foul smell (Soni et al., 2013). A variety of therapeutic properties have been attributed to the *P. foetida* plant parts in local medicine because they are rich in bioactive compounds that contain a variety of secondary metabolites including paederolone, paederone, carotene, vitamin C, keto-alcohol, alkaloid, asperuloside, beta-sitosterol and lupeol (Shrreedhara et al., 2011; Soni et al., 2013). Crude extract of *P. foetida* has been shown to have significant antibacterial activity against microorganisms including two Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and three Gram-negative (*Escherichia coli*, *Salmonella typhimurium* and *Shigella flexneri*) bacteria (Uddin et al., 2007). It has been reported that ethanolic extracts from the leaves of *P. foetida* showed antimicrobial activity against *Bacillus subtilis* and *S. aureus* with zones of inhibition ranging from 4 to 12 mm and the minimal inhibitory concentration (MIC) test of the ethanolic extract against *B. subtilis* and *S. aureus* was observed as 1.25 mg/mL (Upadhyaya, 2013). In addition, Morshed et al. (2012) determined the antibacterial activities of n-hexane, chloroform and ethyl acetate fractions of methanolic extracts of whole *P. foetida* plants were screened against various pathogenic microorganisms using the disc diffusion method. The results showed that the methanol extract of the whole plant had no antimicrobial activity, but the ethyl acetate, chloroform and n-hexane fractions exhibited moderate-to-less activity against some organisms tested compared to an antibiotic (kanamycin). The efficacy of *P. foetida* plant extracts against pathogens for medicinal purposes has been demonstrated by several researchers, but beneficial effects in agriculture are still unknown. Although *P. foetida* is used as a traditional medicinal plant, it is also a perennial weed of plantation crops in Thailand (Konvipasruang et al., 2012). Research of *P. foetida* plant extracts to control phytopathogenic bacteria is an interesting alternative to plant protection. Therefore, the objectives of this study were to determine the *in vitro* antibacterial activity of crude extracts from different parts of *P. foetida* against phytopathogenic bacteria and to undertake their phytochemical analysis.

Materials and Methods

Preparation of plant extracts and phytopathogenic bacteria culture

Plant collection

P. foetida during the vegetative stage was collected locally from nearby areas of Mueang district in Maha Sarakham province, Northeastern Thailand in October 2017. The whole samples were thoroughly washed using tap water. The leaves, roots and stems of *P. foetida* were separated and air-dried at room temperature for 3–4 hr and finally dried in a hot-air oven at 45–50°C for 1 d or until dry. The dried plant samples were ground using a small grinder into fine powder and weighed. Then the dried plant powder samples were kept at 4°C in sealed plastic bags until analysis.

Plant extraction

The powder samples from different parts of *P. foetida* were prepared at ratios of 1:1 and 1:1:1 (weight per weight) as follows: leaves (L), stem (S), roots (R), leaves mixed with stem (L+S), leaves mixed with roots (L+R), stem mixed with roots (S+R), and leaves and stem mixed with roots (L+S+R). All mixes were extracted using 95% ethanol by following the maceration method according to Bussaman et al. (2012). Plant samples were then soaked in ethanol 95 % (ratio between plant powder and solvent was 1:3 weight per volume) and the mixtures were then agitated for 48 hr on a rotary shaker at 150 revolutions per minute (rpm) at room temperature. The obtained extracts were filtered through Whatman filter paper no.1 and were transferred into 250 mL round-bottomed flasks. The extract solutions were evaporated using a rotary evaporator at 45°C to concentrate the extracts. Finally, the concentrated extracts were allowed to dry in a hot-air oven, weighed again and kept at 4°C until analysis.

Bacterial preparation

The phytopathogenic bacteria, consisting of *Xanthomonas campestris*, *Ralstonia solanacearum* and *Erwinia carotovora* were obtained from the Plant Protection research and Development Office, Department of Agriculture, Bangkok, Thailand. *X. campestris*, *R. solanacearum* and *E. carotovora* are bacterial pathogens that cause leaf spot disease in tomato and crucifers, wilt of banana, tomato and potato and soft rot disease of melon and potato disease, respectively (El-Hendawy et al., 1998; AL-Saleh, 2011; Biswal, 2015). Pure cultures were maintained at 30°C in nutrient agar (NA). The phytopathogenic bacteria for antibacterial activity were prepared by inoculating into Nutrient broth and incubated at 30°C and 150 rpm agitation on a rotary shaker for 16–18 hr (log phase, growth curve data not shown) to obtain bacterial suspensions of 5.0×10^8 to 8.0×10^8 colony forming units (CFU)/mL and diluted to attain a viable cell count of 1×10^6 CFU/mL by the viable count method (serial dilution, Bussaman et al., 2015) for the antibacterial activity test.

Antibacterial activity of crude plant extracts

The agar disc diffusion method (Mostafa et al., 2017) was used to evaluate the antibacterial activity of the seven crude plant extracts. All crude extracts were re-dissolved in dimethyl sulfoxide to obtain a final concentration of 100 mg/mL. A sample of 15 mL of NA medium was poured into separate sterile Petri dishes followed by 0.1 mL of phytopathogenic bacteria suspension previously prepared was spread on the surface NA on the agar plate. Sterile filter paper discs loaded with 15 µL of crude plant extract concentration at 100 mg/mL were placed on top of the NA agar plates. A filter paper disc loaded with streptomycin was used as the positive control. All treatments were incubated at 30°C for 24 hr. The presence of clear zones was measured and considered as an indication of antibacterial activity.

Determination of minimum inhibitory concentrations of crude plant extracts

MIC is defined as the lowest concentration of the crude plant extract that inhibits the bacteria growth after 24 hr of incubation. The crude extracts of *P. foetida* that had the highest antibacterial activities (strong inhibition was based on the section of antibacterial activity of crude extracts at 100 mg/mL with the largest clear zone) were selected for MIC assay. The selected crude plant extracts were prepared at various concentrations of 150 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.15 mg/mL and *in vitro* testing was applied against the phytopathogenic bacteria using the agar dilution method modified from Thong-on et al. (2013). Briefly, the preparations of selected crude plant extract at various concentrations were mixed with crude plant extracts with NA medium in Petri dishes using a total volume per dish of 15 mL (with NA medium as diluent). Each NA plate with the most effective crude plant extract at various concentrations was followed with 2 µL of phytopathogenic bacteria suspension (1×10^6 CFU/ mL). All treatments were incubated at 30°C for 24 hr. The MIC value was measured based on observation of bacterial colony growth and recorded against the concentrations of the effective crude plant extracts.

Determination of minimum bactericidal concentrations of crude plant extracts

Phytopathogenic bacteria were chosen from the lowest concentrations of the crude plant extract plates exhibiting minimal growth (from bacterial colony growth observation of MIC plates) for streak plating onto sterile NA plates using the technique of Thong-on et al. (2013). The plates were incubated at 30°C for 24 hr. The minimal bactericidal concentration (MBC) was taken as the concentration of plant extracts that did not exhibit any bacterial growth on the freshly inoculated agar plates.

Phytochemical analysis

The components and contents of phenolic acids and flavonoids from the crude extracts were determined using high performance liquid chromatography (LC-20AC, Shimadzu; Tokyo, Japan) according to the method of Kaewseejan et al. (2015). Each crude extract was dissolved in ethanol, filtered through a 0.45 µm membrane filter and injected onto an Inetsil ODS-3 C18 column (4.6 mm × 250 mm, 5 µm; Hichrom Limited, Reading, UK) with an injection volume of 20 µL. The mobile phases used were 1 % acetic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. The components of phenolic acid and flavonoid in the extracts were separated using gradient elution at 38°C. The gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5% to 9% solvent B; from 5 to 15 min, 9 % solvent B; from 15 to 22 min, linear gradient from 9% to 11% solvent B; from 22 to 38 min, linear gradient from 11% to 18% solvent B; from 38 to 43 min, linear gradient from 18% to 23% solvent B; from 43 to 44 min, linear gradient from 23% to 90% solvent B; from 44 to 45 min, linear gradient from 90% to 80% solvent B; from 45 to 55 min, isocratic at 80 % solvent B; from 55 to 60 min, linear gradient from 80% to 5% solvent B; and a re-equilibration period of 5 min with 5% solvent B used between individual runs. The eluted phenolic compounds were detected at 280 nm for phenolic acids and at 370 nm for flavonoids using a UV-diode array detector (SPD-M20A; Shimadzu; Tokyo, Japan). Phenolic compounds in the extracts were identified by a direct comparison of their retention times and their UV spectra with those of standard compounds. The contents of the phenolic acid and flavonoids were calculated based on the linear regression equations ($y = ax + b$) from the external calibration curves of the standards. The calibration curves were determined by plotting the peak area (y axis) against concentrations of standard compounds (x axis) in the range 6.25–100 µg/mL, and the linear responses with correlation coefficients higher than 0.99 were obtained.

Statistical analysis

All data were subjected to analysis of variance. The means of clear zones of all treatments were compared and significance was determined using Duncan's test at the 0.05 level of significance.

Results and Discussion

Antibacterial activity of crude plants extract

Seven crude plant extracts of *P. foetida* were investigated to evaluate their antibacterial activity against three strains of phytopathogenic bacteria (*X. campestris, R. solanacearum* and *E. carotovora*) using the agar disc diffusion method. Evaluation of antibacterial activity of these plant extracts is recorded in Fig. 1. The results revealed similar effects of crude extracts from the different part of *P. foetida* at a concentration of 100 mg/mL on the antibacterial activity against the phytopathogenic bacteria. The crude extracts from the leaves and L+S+R produced effective inhibition of the

phytopathogenic bacteria. The crude extract from whole parts of the *P. foetida* plant (L+S+R) had the highest antibacterial activity (clear zone) against *X. campestris* and *R. solanacearum* with diameters of 10 mm and 10.33 mm, respectively (Figs. 1A and 1C). Crude leaf extract of *P. foetida* had the highest antibacterial activity against *E. carotovora* with a 9 mm diameter clear zone followed by crude extracts from the stem (8.67 mm) and root (8.50 mm), which were not significantly different from all crude extracts (Fig.1B). The other crude extracts of *P. foetida* had antibacterial activities in ranges between 7.50–9.50 mm, 8.33–9.33 mm and 6.50–7.50 mm for *X. campestris*, *R. solanacearum* and *E. carotovora*, respectively. Standard streptomycin (25 mg/mL) had a clear zone of 20–23.5 mm. The current study has provided the first report on the effectiveness of crude *P. foetida* extracts against phytopathogenic bacteria (*X. campestris*, *R. solanacearum* and *E. carotovora*). Uddin et al. (2007) reported that the ethanol extract of *P. foetida* had strong antibacterial activity against *Shigella flexne*ri and *Enterococcus faecalis* (clear zones with diameters of 17 mm and 18 mm, respectively) and moderate activity against *E. coli* and *S. aureus* (clear zones with diameters of 17 mm and 15 mm, respectively) at a concentration of 25 mg/mL. Morshed et al. (2012) found that the n-hexane fraction of whole plants (300 µg/disc) had moderate antibacterial activity for two Gram-positive bacteria (*Bacillus cereus* with a clear diameter of 12 mm and *S. aureus* with a clear dimeter of 14 mm) and two Gram-negative strains (*E. coli* with a clear zone diameter of 18 mm and *Vibrio mimicus* with a clear diameter of 16 mm). Previous studies reported that the crude extract of *P. foetida* had significant antibacterial activity against *S. flexneri*, *S. aureus*, *E. coli* and *E. faecalis*. The results of the experiment clearly indicated that the *P. foetida* plant could be used for its antibacterial activity (Uddin et al., 2007; Soni et al., 2013). The results indicated that the highest antibacterial activity was found in the crude extract of whole parts of the *P. foetida* plant except for *E. carotovora* where the highest antibacterial activity was in *P. foetida* crude extracts from the leaf but this was not significantly different from the crude extract of the stem and roots. Hence, selected crude extract of whole parts of the *P. foetida* plant (R+S+L) were tested to determine their MIC and MBC values against the phytopathogenic bacteria.

Minimum inhibitory concentrations of effective crude plants extract

The MIC values of the crude extract of whole parts of the *P. foetida* plant were determined using the agar dilution method to evaluate their bacteriostatic properties. The concentration effect of the effective plant extracts is reported in Tables 1 and 2 The inhibitory effect of the *P. foetida* extract started at 3.15 mg/mL for *E. carotovora*, was 6.25 mg/mL for *X. campestris* and *R. solanacearum* with minimal bacterial colony growth (the cell concentration was approximately 1×10^4 CFU/mL). These results suggested that *E. carotovora* was the most susceptible strain to crude plant extract. 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 ethanolic *P. foetida* extract exhibited no inhibitory effect against *Proteus vulgaris*, *E. coli* and *Pseudomonas auroginosa* at MIC test. The ethanolic *P. foetida* extract in the current study had strong antibacterial activity based on the MIC value against Gram-negative bacteria and thus suggested that different localities and environmental factors exposed by *P. foetida* may influence the results. Upadhyaya and Saikia (2012) described plant extracts of *Centella asiatica* L. from different localities of Assam, India with different phenolic and nutrient contents and different organic manure regimes that had significant effects on the phenolic content of *Adhatoda vasica* leaves. Furthermore, Oloumi and Hassibi (2011) reported that temperature and soil factors were the most important factors affecting the secondary metabolite content in roots of *Glycyrrhiza glabra* plants.

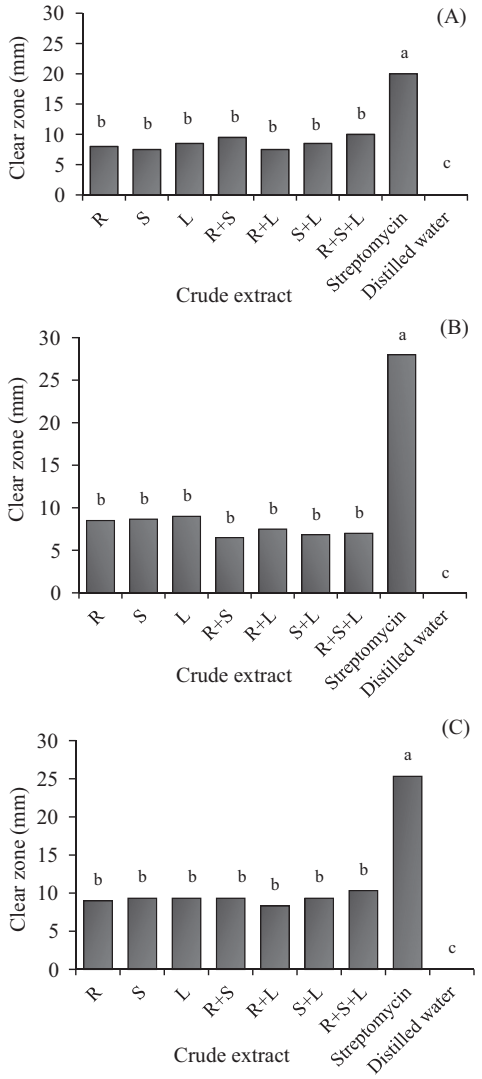

Fig. 1 Antibacterial activity of phytopathogenic bacteria of crude extracts: (A) *X. campestris;* (B) *E. carotovora*; (C) *R. solanacearum*, where bars (mean values) with the same letter are not significantly different by Duncan's test at $p \leq 0.05$ and R = root, S = stem, L = leaf.

Table 1 Minimum inhibitory concentration of the most effective crude *P. foetida* plant extracts against phytopathogenic bacteria.

Phytopathogenic bacterium	Crude extract (mg/mL)							
	150	100	50	25	12.5	6.25	3.15	NA
X. campestris	–	–	–	–	–	+	++	+++
E. carotovora	–	–	–	–	–	–	+	+++
R. solanacearum	–	–	–	–	–	+	++	+++

– = no bacterial colony growth; + = minimal bacterial colony growth (~ 1 ×10^4 colony forming units (CFU)/mL); ++ = moderate bacterial colony growth (~1 × 10^5 CFU/mL); +++ = maximal bacterial colony growth (~1 × 10^6 CFU/mL where all treatments were compared with Nutrient agar (NA).

Table 2 Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of the most effective crude *P. foetida* plant extracts against phytopathogenic bacteria.

Phytopathogenic bacterium	MIC (mg/mL)	MBC (mg/mL)
X. campestris	≥ 6.25	25.00
E. carotovora	≥ 3.15	6.25
R. solanacearum	≥ 6.25	12.50

Minimum bactericidal concentrations of effective crude plant extracts

The MBC was confirmed by the absence of bacterial growth of the tested strains streaked from minimal bacterial colony growth corresponding to their lowest MIC value. The crude extract of *P. foetida* had effective bactericidal activity against the tested phytopathogenic bacteria with MBC values of 25 mg/mL, 6.25 mg/mL and 12.50 mg/mL for *X. campestris*, *E. carotovora* and *R. solanacearum*, respectively. *E. carotovora* which was the most sensitive to crude extract (6.25 mg/mL) followed by *R. solanacearum* and *X. campestris*, respectively. The MIC and MBC values of the effective crude plant extracts suggested that *P. foetida* can be used to control and protect crops from plant pathogenic bacteria. The bacterial strains included in this study were chosen for their importance in plant disease. The phytopathogenic bacteria *X. campestris*, *E. carotovora* and *R. solanacearum* are considered as some of the most common sources of bacterial disease, including bacterial leaf spot disease in tomato (Al-Saleh, 2011), *Xanthomonas* and *Ralstonia* strain wilt of banana (Blomme et al., 2017), soft rot disease in melon and soft rot erwinias in potato disease is caused by *E. carotovora* (El-Hendawy et al., 1998; Pérombelon et al., 2002).

Analysis of phytochemical compounds

The phenolic and flavonoid contents were estimated in the crude leaf extracts of *P. foetida*, expressed in milligrams per gram of crude extract. The phenolics acid content and flavonoids content of the crude extracts varied in the ranges 0.01–0.40 mg/g crude extract and 0.85–113.68 mg/g crude extract, respectively. Ferulic acid and luteolin were the major components of the *P. foetida* crude extract, which possibility acted as bioactive compounds to inhibit bacterial growth (Tables 3 and 4). The crude extract of *P. foetida* (whole plant) consisted mainly of phenolics and flavonoids composed of ferulic acid and luteolin, which are known for their strong antioxidant, antibacterial and anti-fungal activity (Ikewuchi et al., 2013). The antimicrobial activity of luteolin had complex suppressive effects concerning the processes of adherence to epithelial cells (Yordanov et al., 2008) and it could inhibit the activity of DNA topoisomerase I and II, which resulted in some decrease in nucleic acid and protein synthesis (Wang and Xie, 2010). The crude extract of *P. foetida* contains different types of secondary compounds that have different structural characteristics with different antibacterial effects. Different compounds such as phenolic compounds have successfully proven themselves as antioxidants, which is of great importance in the current study as a good number of active ingredients have been extracted from this plant and used in agriculture. The presence of a good amount of phenolic, flavonoid and antimicrobial activity has justified the use of the bioactive extract from the plant as a bacterial agent for biological control.

Table 3 Phenolic acid content of crude extract of whole parts of *P. foetida*

Phenolic compound	Individual phenolic acid content in extract (mg/g crude extract)
Gallic acid	0.11 ± 1.45
Protocatechuic acid	0.18 ± 1.50
p-Hydroxybenzoic acid	0.17 ± 0.85
Chlorogenic acid	0.01 ± 0.05
Caffeic acid	0.03 ± 0.09
Syringic acid	0.05 ± 1.02
p-Coumaric acid	0.13 ± 1.42
Ferulic acid	0.40 ± 0.98
Sinapic acid	0.17 ± 0.56

Table 4 Flavonoid content of crude extract of whole parts of *P. foetida*

Flavonoid compound	Individual flavonoid content in extract (mg/g crude extract)
Rutin	1.03 ± 0.45
Myricetin	0.85 ± 0.00
Luteolin	113.68 ± 1.85
Quercetin	1.76 ± 0.05
Apigenin	2.67 ± 0.09
Kaempferol	11.17 ± 0.02

The anti-phytopathogenic bacterial activity of *P. foetida* crude extracts against the Gram-negative bacteria, *X. campestris*, *E. carotovora* and *R. solanacearum* was similar in effect to other research reported. Similar results have also been stated in previous studies that the leaf extract of *Jasminum officinale* was highly effective against 25 strains of *X. campestris* pv. *mangiferaeindicae* (the inhibition zone was in the range 20.27–21.94 mm) (Pawar, 2015). According to Thirumalesh et al. (2012), petroleum ether crude extract of *Sapindus laurifolia* was highly effective against *X. campestris* pv. *mangiferaeindica*e (inhibition zone diameter 15 mm) and low MIC and MBC values at 2 µg/mL. Additionally, Viswanath et al. (2018) reported that leaf

extract of *Datura stramonium* (Jimsonweed) had the best control with the highest mean diameter zone of inhibition of 11.70 mm against *E.carotovora* causing storage soft rot of potato and showed potential for control of the disease *in vivo*. Moreover, it has been reported that 20 different plant extracts displayed antibacterial activity against *R. solanacearum* (inhibition zone ranging from 6.68 to 10.00 mm in diameter), while the leaf extract of *Musa paradisica* and the flower extract of *Nerium indicum* had the largest zones of inhibition (10.00 mm) against *R. solanacearum* causing bacterial wilt and brown rot of potato (Biswal, 2015). Furthermore, Ooshiro et al. (2004) reported that 70% ethanolic extract of *Geranium carolinianum* L. had strong antibacterial activity against *R. solanacearum* under both *in vitro* and field test conditions. Further study demonstrated that ethyl acetate extracts of leaves of *Ipomoea batatas* and *Brassica oleracea* at concentrations of 0.4 mg/mL and 0.05 mg/mL, respectively, had the best antibacterial activity against *R. solanacearum* and produced the highest inhibition zones of 10.20 mm and 10.12 mm diameter, respectively (Wagura et al., 2011). Nonetheless, the current findings are the first to indicate the effectiveness of *P. foetida* extract against phytopathogenic bacteria and to suggest it could be used as an alternative source of antibacterial agents for protection of crops against bacterial infection.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

AL-Saleh, M.A. 2011. Pathogenic variability among five bacterial isolates of *Xanthomonas campestris* pv. *vesicatoria*, causing spot disease on tomato and their response to salicylic acid. J. Saudi Soc. Agric. Sci. 10: 47–51.

Biswal, G. 2015. Studies on antibacterial activity of some aqueous plant extracts against *Ralstonia solanacearum* causing bacterial wilt and brown rot of potato. Int. J. Plant Sci. 10: 1–6.

Blomme, G., Dita, M., Jacobsen, K.S., Pérez Vicente, L., Molina, A., Ocimati, W., Prior, P. 2017. Bacterial diseases of bananas and enset: Current state of knowledge and integrated approaches toward sustainable management. Front. Plant Sci. 8: 1–25.

Bussaman, P., Namsena, P., Rattanasena, P., Chandrapatya, A. 2012. Effect of Crude Leaf Extracts on *Colletotrichum gloeosporioides* (Penz.) Sacc. Psyche. (Camb Mass). 2012: 1–6.

Bussaman, P., Rattanasena, P., Namsena, P. 2015. Antimicrobial activities of some local plants of Thailand against acne-producing bacteria. FABJ. 3: 184–192.

El-Hendawy, H.H., Zeid, I. M., Mohamed, Z.K. 1998. The biological control of soft rot disease in melon caused by *Erwinia carotovora* subsp. *carotovora* using *Pseudomonas fluorescens*. Microbiol. Res. 153: 55–60.

Fawcett, C.H., Spencer, D.M. 1970. Plant chemotherapy with natural products. Annu. Rev. Phytopathol. 8: 403–418.

Ikewuchi, J.C., Ikewuchi, C.C., Ifeanacho, M.O. 2013. Analysis of the phytochemical composition of the leaves of *Chromolaena odorata* King and Robinson by gas chromatography-flame ionization detector. Pac. J. Sci. Technol. 14: 360–378.

Kaewseejan, N., Sutthikhum, V., Siriamornpun, S. 2015. Potential of *Gynura procumbens* leaves as source of flavonoid-enriched fractions with enhanced antioxidant capacity. J. Funct. Foods. 12: 120–128.

Konvipasruang, P., Chandrapatya, A., Amrine, J. W., Ochoa, R., Bauchan, G., Pratt, P. 2012. A new species, *Aceria neopaederiae* (Acari: Eriophyidae), infesting *Paederia foetida* L. (Rubiaceae) in Thailand, Hong Kong and Singapore. Syst. Appl. Acarol-UK. 17: 191–202.

Latha, P., Anand, T., Ragupathi, N., Prakasam, V., Samiyappan, R. 2009. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. Biol. Control. 50: 85–93.

Morshed, H., Islam, M., Parvin, S., Ahmed, M.U., Islam, M.S., Mostofa, A.G.M., Sayeed, M.S.B. 2012. Antimicrobial and cytotoxic activity of the methanol extract of *Paederia foetida* Linn. (Rubiaceae). J. Appl. Pharm. Sci. 2: 77–80.

Mostafa, A.A., Al-Askar, A.A., Almaary, K.S., Dawoud, T.M., Sholkamy, E.N., Bakri, M.M. 2017. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J. Biol. Sci. 25: 361–366.

Oloumi, H., Hassibi, N. 2011. Study the correlation between some climate parameters and the content of phenolic compounds in roots of *Glycyrrhiza glabra*. J. Med. Plant Res. 5: 6011–6016.

Ooshiro, A., Takaesu, K., Natsume, M., Taba, S., Nasu, K., Uehara, M., Muramoto, Y. 2004. Identification and use of a wild plant with antimicrobial activity against *Ralstonia solanacearum*, the cause of bacterial wilt of potato. Weed Biol. Manag. 4: 187–194.

Pawar, B.T. 2015. Determination of antibacterial activity of leaf extracts of *Jasminum officinale* against *Xanthomonas campestris* pv. *mangiferaeindicae*. Am. J. Adv. Drug Deliv. 3: 18–23.

Pérombelon, M.C.M. 2002. Potato diseases caused by soft rot erwinias: An overview of pathogenesis. Plant Pathol. 51: 1–12.

Shrreedhara, C.S., Udupa, N., Shetty, S. 2011. Quantification of phytoconstituents of the leaves of *Paederia foetida* by HPTLC. In: proceedings of 21st International Symposium for High Performance Thin Layer Chromatography HPTLC. Basel, Switzerland.

Soni, R.K., Irchhaiya, R., Dixit, V., Alok, S. 2013. *Paederia foetida* Linn: Phytochemistry, pharmacological and traditional uses. Int. J. Pharm. Sci. Res. 4: 4525–4530.

Stephan, D., Schmitt, A., Carvalho, S.M., Seddon, B., Koch, E. 2005. Evaluation of biocontrol preparations and plant extracts for the control of *Phytophthora infestans* on potato leaves. Eur. J. Plant Pathol. 112: 235–246.

Thirumalesh, B.V., Thippeswamy, B., Krishnappa, M. 2012. *In vitro* efficacy of eight plant extracts on *Xanthomonas campestris* pv. *mangiferaeindicae* affecting bacterial black spot of mango. J. Crop. Sci. 3: 78–80.

Thong-on, R., Phumalee, K., Akanithaphichart, P., Chantama, S.S. 2013. Antibacterial activities of herbal extracts from *Euphobia hirta* and *Euphobia thymifolia*. Journal of Science and Technology, Ubon Ratchathani University. 15: 41–47.

Uddin, B., Nahar, T., Khalil, M.I., Hossain, S. 2007. *In vitro* antibacterial activity of the ethanol extract of *Paederia foetida* L. (Rubiaceae) leaves. J. Life Sci. 19: 141–143.

Upadhyaya, S. 2013. Screening of phytochemicals, nutritional status, antioxidant and antimicrobial activity of *Paederia foetida* Linn. from different localities of Assam, India. J. Pharm. Res. 7: 139–141.

Upadhyaya, S., Saikia, L.R. 2012. Evaluation of phytochemicals, antioxidant activity and nutrient content of *Centella asiatica* (L.) urban leaves from different localities of Assam. Int. J. Pharma Bio Sci. 3: 656–663.

Viswanath, H.S., Bhat, K.A., Bhat, N.A., Wani, T.A., Mughal, M.N. 2018. Antibacterial efficacy of aqueous plant Extracts against storage soft rot of potato caused by *Erwinia carotovora*. Int. J. Curr. Microbiol. Appl. Sci. 7: 2630–2639.

Wagura, A.G., Wagai, S.O., Manguro, L., Gichimu, B.M. 2011. Effects of selected plants' extracts on in vitro growth of *Rahtonia slanacearum* (Smith), the causal agent of bacterial wilt of Irish potatoes. Plant Pathol. J. 10: 66–72.

Wang, Q., Xie, M. 2010. Antibacterial activity and mechanism of luteolin on *Staphylococcus aureus*. Acta Microbiol. Sin. 50: 1180–1184.

Yordanov, M., Dimitrova, P., Patkar, S., Saso, L., Ivanovska, N. 2008. Inhibition of *Candida albicans* extracellular enzyme activity by selected natural substances and their application in Candida infection. Can. J. Microbiol. 54: 435–440.