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Identification of phenolic compounds and evaluation of antibacterial properties of *Piper sarmentosum* Roxb. against rice pathogenic bacteria

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ABSTRACT

Aims: *Piper sarmentosum* Roxb. has a long history of medicinal usage and potential in treating many diseases and ailments. It is known for its medicinal properties and contains a variety of active chemical compounds. This study was conducted to identify and quantify the phenolic compounds of the leaf and fruit extract of *P. sarmentosum*, as well as their antibacterial activities.
Methodology and results: High Performance Liquid Chromatography (HPLC) was carried out to identify the phenolic compounds in the samples. Antibacterial performance of the samples was measured using agar well and disc diffusion assay, where its MIC values were then determined. After going through HPLC-UV, the major phenolic compounds identified in both extracts were tannic acid, gallic acid, quercetin and naringin. The leaf and fruit of *P. sarmentosum* exhibited moderate to strong antibacterial activity when tested against pythopathogenic bacteria with an inhibition range of 10.67-17.33 mm at 100 mg/mL.
Conclusion, significance and impact of study: The leaf and fruit extracts of *P. sarmentosum* were proven to have effective inhibitory effects on *Pseudomonas fuscovaginae* and *Xanthomonas oryzae* pv. *oryzae*, which are also the causal agents of sheath brown rot and bacterial leaf blight in rice. This is believed to be due to the presence of the phenolic compounds in these samples. In a subsequent study, the researchers are planning to apply a formulation, developed from the crude extract, in the glasshouse and field trial.

Keywords: Antibacterial activity, phenolic compounds, plant crude extracts, *Pseudomonas fuscovaginae*, *Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

Piper sarmentosum is a tropical plant from the Piperaceae family which is commonly consumed as a vegetable and used as a traditional medicine to treat many ailments. It is a wild growing herb with long creeping stems, with heart-shaped and alternating leaves. The young leaves are usually light green and have a waxy surface. The plant produces small, white flowers in the form of spikes and is located at the terminal or leaf opposite spikes (Chaveerach *et al.*, 2008). The fruits are big and taste sweet when ripe. When the fruit matures, it turns black and dry with several rounded bulges with pungent odour. *Piper sarmentosum* have been reported to have various chemical constituents such as naringenin (Subramaniam *et al*., 2003), phenylpropanoids (Masuda *et al.*, 1991), cinnamic acid (Diastutia and Delsy, 2012), amides, pyrones, flavonoids, sterols, and neolignans (Atiax *et al*., 2010).

Piper sarmentosum has various pharmacological effects and has been used for wounds, osteoporosis, anti-nociceptive, anti-inflammatory, and as an antioxidant (Zakaria *et al*., 2010). A recent study provided experimental evidence for the application of *P. sarmentosum* in botanical pesticides (Qin *et al.*, 2010). It has been experimentally claimed to have potential in the treatment of different kinds of diseases, considering its wide application in traditional medicine. For example, many species of *Piper* were used to treat inflammatory diseases in ancient Chinese medicine (Stohr *et al.*, 2001). In Southern Thailand, the liquid mixture of the whole plant of *P. sarmentosum* has been used to treat diabetic patients (Peungvicha *et al*., 1998) and the crude extract has been reported to reduce blood glucose levels of onset diabetic patients (Pongmarutai, 1969).

Additionally, *P. sarmentosum* has also been used as a carminative as well as to relieve coughs and muscle pain. The fruits and leaves of this plant are used as an expectorant (Saralamp *et al*., 1996). The research on *P. sarmentosum* has remarkably increased worldwide with numerous studies showing the immense potential of plants as an antimicrobial agent. Recent studies and researches are focusing on producing plant-based antibiotics, bactericides, and biopesticides. Studies on the

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natural phytochemicals of *P. sarmentosum* claimed that it exhibits antioxidant, antimicrobial, and anti-inflammatory properties (Ao *et al*., 2008; Al-Zubaydi *et al*., 2009). These plant extracts can overcome the harmful effects of chemical pesticides and the resistance of microbes to pesticides. Due to these properties, an investigation for new alternatives in natural products is required. Phenolic acids and flavonoids from plants are categorized under the phenolic group, which are known to exhibit antimicrobial activity and inhibitory actions (Maddox *et al*., 2010).

The detrimental effects of microbes have led to the wide discovery of plants with antimicrobial properties. Subsequently, this has caused an increase in the investigation on plants as a source for disease management, which led researchers to focus on the effectiveness of plant-based natural antimicrobials against harmful pathogenic bacteria. Plant extracts have been proven to have antimicrobial effects and they consist of a variety of phytochemicals. Therefore, a further investigation of their effects in controlling various types of microbes is needed. The identification of the phenolic compounds and antibacterial activities in *P. sarmentosum* extracts have yet to be tested against plant pathogenic bacteria. Thus, this study was conducted to evaluate the potential biological activities of *P. sarmentosum* leaf and fruit extracts by testing it against *P. fuscovaginae* and *X. oryzae* pv. *oryzae*; to evaluate their inhibitory effects on the growth of these bacteria.

MATERIALS AND METHODS

Plant materials

Fresh leaves and fruits of *P. sarmentosum* were collected from the botanical garden of the Institute of Bioscience, Universiti Putra Malaysia (UPM), Serdang, Selangor. The plant species were identified by a botanist, Dr. Shamsul Khamis, from the Institute of Bioscience, UPM and the voucher specimen (SK2171/13) was deposited in the Herbarium, Biodiversity Unit, Institute of Bioscience, UPM. The leaves were washed, rinsed with sterile distilled water, and dried in room temperature. They were then crushed into fine powder using a commercial blender chopper (ZK100, Braun) with a fine mesh strainer of 0.5 mm.

Extract preparation

The extraction of the phenolic compounds from *P. sarmentosum* was conducted according to the method of Singh *et al*. (2010) with some modifications. The plant tissue generally starts secreting phenolic compounds after it is collected from the plants (Maddox *et al*., 2010). One gram of the dried powder of *P. sarmentosum* leaf and fruit were macerated and suspended in 5 mL of 80% (v/v) methanol. The sample was then subjected to centrifugation at 12,500 ×*g* for 15 min. The clear supernatant was collected and subjected to charcoal treatment. The residue of the extraction was re-extracted twice with the same extracting methanol and the supernatant was then pooled together and evaporated using a rotary evaporator with a water bath at 35 °C (R-215, Buchi). The residue obtained was re-dissolved in 1.0 mL of methanol (HPLC grade) and filtered through a 0.45 µm (Millipore) filter. Aliquots (10 µL) of this diluted solution were injected into HPLC.

Determination of total phenolic content of the plant extracts.

The total phenolic content of the samples was measured using a modified version of the Folin-Ciocalteu assay (Singleton and Rossi, 1965). The leaf and fruit sample extract (0.5 mL of 1 mg/mL) was individually mixed with 5mL of Folin-Ciocalteau reagent of 1:10 dilution with distilled water for 5 min. Next, 4 mL of aqueous Na_2CO_3 with 1 M concentration were added and the mixture was set aside for 1 h. The phenol contents of the samples were determined using calorimetry at 765 nm. Finally, the results were presented as micrograms of Gallic acid per gram of dry sample (µg GAE/g DS).

Determination of the phenolic compounds using HPLC

The phenolic compounds of the leaf and fruit of *P. sarmentosum* were quantitatively measured by a reversed phase HPLC technique according to the method of Mira *et al*. (2008) and Singh *et al*. (2010) and The wavelength selected for the detection of phenolic acid was 280 nm and the column oven temperature was set to 35 °C. The phenolic acid standards (Sigma Chemicals Co., St Louis, MO, USA) used for the detection and identification of the peaks consisted of caffeic acid, gallic acid, tannic acid, p-coumaric acid, ferulic acid, quercetin, rutin, and naringin. With the eluent flow rate set to 1.0 mL/min, 40 µL of the extract was injected into the HPLC analytical column (Ascentis® C18 60Å 5 µm 4.6 × 150 mm, Supelco, Sigma-Aldrich, USA) by an auto injector. The running conditions, in a mobile phase (filtered under vacuum through a 0.45 µm membrane before use), were as the following: A: contained 1% trifluoroacetic acid; B: methanol (80:20 v/v). The elution comprised of the following gradient: 80:20 A: B for 5.0 min ramped to 50:50 over 5–7 min, then set to a constant ratio of 30:70 for 7–20 min. The column was then washed with 100% acetonitrile for 30 min and re-conditioned with 20% acetonitrile for 20 min between runs. Each standard peak was recorded and stored in the HPLC spectrum library. The identification of *P. sarmentosum* compounds were done by comparing the retention time and the spectra of unknown compounds with those in the HPLC library of standards. The purity of the peaks was determined to ensure accuracy of identification. For close peaks, an integration program in the HPLC software was used to split them and data was for calculations. Quantification of the phenolic acids contained in the extracts was carried out by an external standard method. The concentration selected for identifying phenolic acid in all samples was

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0.1%, whereas the calibration curves were constructed at 10, 50, 100, 250, 500, 1000 µg/mL. The calibration curves were determined by plotting the phenolic acid peak at 280 nm against the phenolic acid standard. The data was analysed through linear regression.

Antibacterial activity

Bacteria cultures

Registered bacteria isolates, namely Gram-negative bacteria *P. fuscovaginae* T1 strain (Accession number JX915743.1) and *X. oryzae* (Accession number CP000967.1), were obtained from the Microbiology Laboratory, Department of Plant Protection, Universiti Putra Malaysia, Selangor, Malaysia. Both cultures were maintained on Peptone-Sucrose Agar and King's B media, respectively, at 4 °C for continuous viability and were subcultured regularly.

Bacterial susceptibility testing

The antibacterial activity exhibited by the *P. sarmentosum* leaf and fruit extracts were observed using agar well diffusion assay (Egharevba *et al*., 2010) and disc diffusion assay (Hendra *et al.*, 2011). First, the test bacterium was inoculated into 10 ml of Mueller Hinton Broth (MHB) in a test tube and was vortexed well. Next, the inoculation was placed on a rotary shaker for 24 h at 28 °C. The optical density (OD) was measured with a spectrophotometer and the density was adjusted to 0.1 (10^8 cell population) at 660 nm. Finally, the inoculation was plated out as inoculums (Taweechaisupapong *et al*., 2010).

Agar well diffusion assay

The culture media used for this assay was Muller Hinton Agar (MHA). Previously seeded with standardized bacterium, six wells with diameters of 4 mm each were made on the MHA plate using a sterile cork borer. Then, a standard antibiotic and dilution of aqueous methanol extracts were filled into the wells and incubated at 28 °C for 24 h. The wells were filled with 50µl of extract at concentrations of 100 mg/mL and control. Aqueous methanol (80:20; v/v) served as the negative control while streptomycin sulfate (30 µg/mL) was used as positive control. The zones of inhibition produced around the wells after incubation were measured using a transparent ruler. The test was assayed on triplicate agar medium plate.

Disc diffusion assay

Firstly, the standardized bacterium (50 µL) was spread over sterile Mueller Hinton agar plates. Paper discs (6 mm) saturated with leaf and fruit extracts of *P. sarmentosum* (10 µL, 100 ppm) were then placed on the media. Next, the plates were left for 30 min at room temperature to allow for the diffusion of the extracts. The plates were then incubated at 28 °C for 24 h and the zones of inhibition were observed. The positive control, streptomycin sulfate (30 µg/mL) was used as a reference control to assess the susceptibility of tested strains. The tests were done in triplicates.

Determination of MIC and MBC of the crude extracts

The MIC of the extracts was determined by macro broth dilution method through a twofold serial dilution. From the *in vitro* assay, the MIC assay was taken from the lowest concentration of the effective extracts that is able to inhibit the bacteria. 25 mg/mL of the assay was taken as the lowest concentration; is the start point for MIC determination. Eleven test tubes with caps were filled with 1 mL Mueller Hinton Broth (MHB) (Oxoid) and autoclaved (Hiclave HVE-50, Hirayama) at 121 °C for 20 min. A 50 mg/mL of *P. sarmentosum* fruit extract was filled into the first test tube to make up the concentration of 25 mg/mL. Then, a two-fold serial dilution was performed from test tube no.1 to test tube no. 10. Test tube no. 11 was left with only MHB without any extract dilution. The bacterial optical density was maintained throughout the experiment at 0.1 $OD_{660.}$ 100 µL of microbial suspension was added into each test tube except for test tube no.10 and mixed well. Test tube no.10, which contains microorganisms cultured in broth with the extract, served as a positive control. Test tube no. 11, containing the mixture of broth and the microorganisms, is the negative control. Then, 50 µL of 2, 3, 5-triphenyltetrazolium chloride (TTC, 2 mg/mL) (Sigma) aqueous solution was added into all test tubes as a dye to indicate bacterial growth. It was incubated for 24 h at 28 °C. The MIC value is indicated as the lowest extract concentration that showed no colour changes (Basri *et al*., 2011). The same procedure was conducted for the fruit extract.

The minimum bactericidal concentration (MBC) value was determined by sub culturing from the wells that showed no colour changes on the sterile MHA plate. It was incubated overnight at 28 °C. The lowest concentration that showed no visible growth on the agar plates was considered as the MBC value (Taweechaisupapong *et al*., 2010).

Statistical analysis

The results were analysed using statistical software JMP (9.0). Data was expressed as MEAN ± STD of triplicate samples by one-way analysis of variance (ANOVA) using Tukey's HSD. In terms of significant difference, $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Phenolic compounds analyses

The presence of phenolic compounds in *P. sarmentosum* leaf and fruit were determined by RP-HPLC. Figure 1 (a), (b), (c) and (d) shows the HPLC chromatogram of the standard phenolic acids used for the identification of the compounds in the extracts. Four standard phenolics were analysed in order to determine their retention times under

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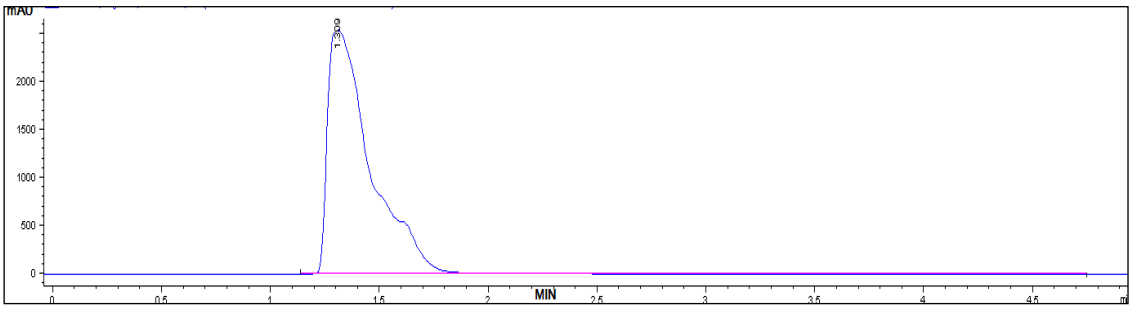
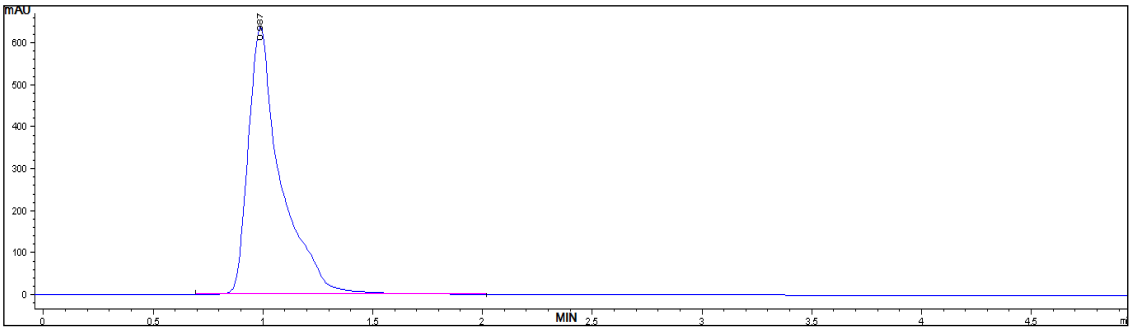


(a) Gallic acid



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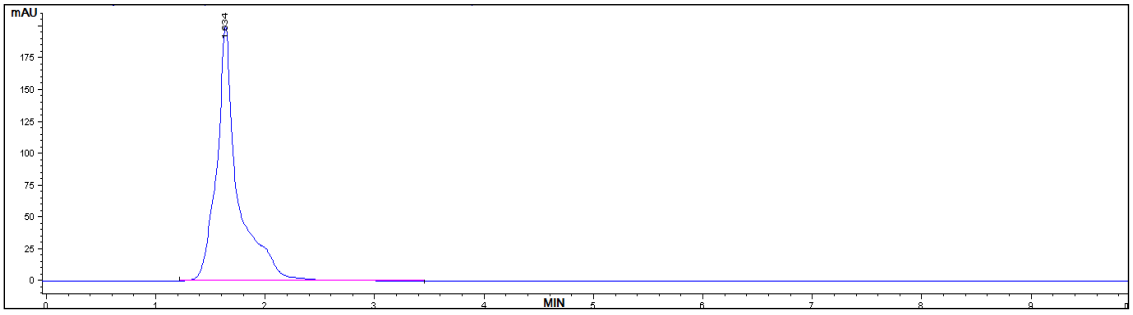
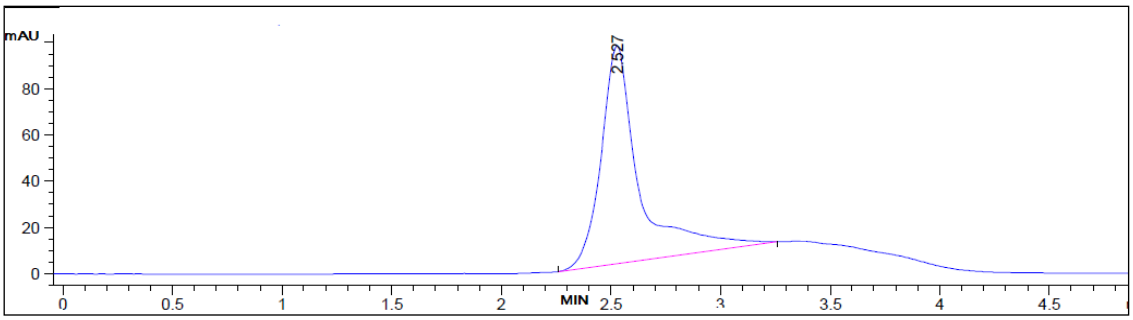


(c) Quercetin



(d) Naringin

Figure 1: Schematization of the HPLC chromatogram showing the four standard phenolic acids (a) gallic acid, (b) tannic acid (c) quercetin, (d) naringin and their respective retention times used for identification of these compounds in the extracts.

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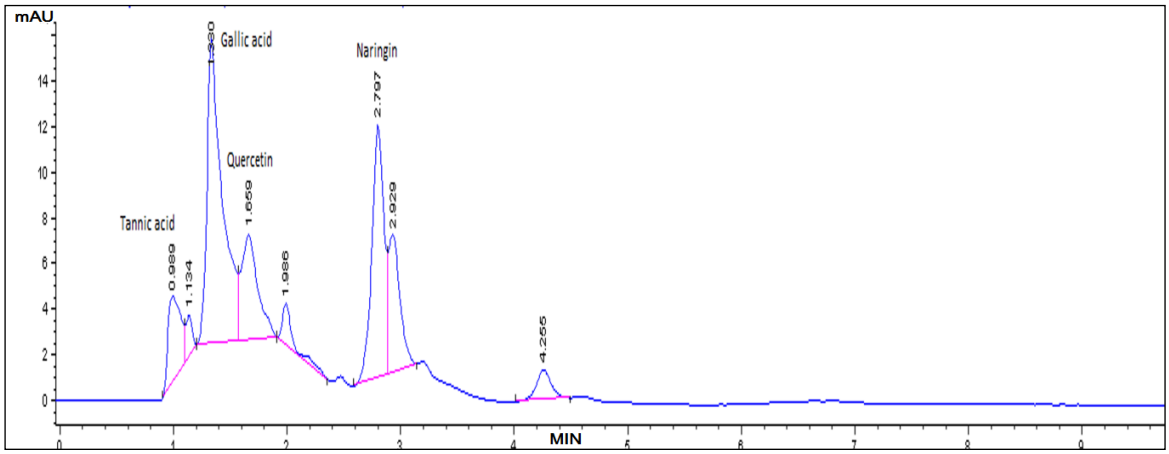

Figure 2: Phenolics content of leaf extract of *P. sarmentosum* analyzed by HPLC at the wavelength of 280 nm.

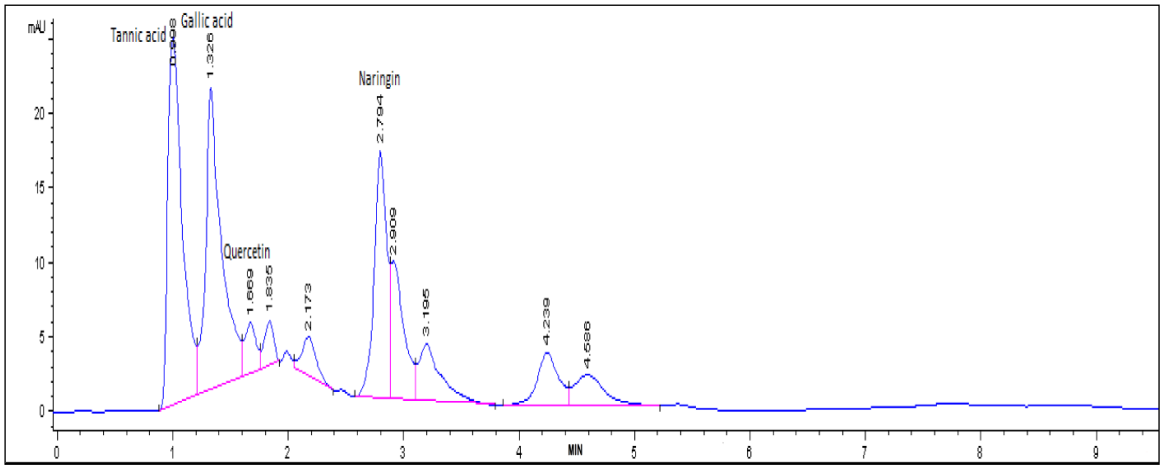

Figure 3: Phenolics content of fruit extract of *P. sarmentosum* analysed by HPLC at the wavelength of 280 nm.

Table 1: Content of phenolics compounds in leaf and fruit of *P. sarmentosum*.

Sample	Phenolics content (µg/g DW)							
	Caffeic acid	Gallic acid	Tannic acid	Ferulic acid	p-coumaric acid	Naringin	Rutin	Quercetin
Leaf	-	22.5	43.1	-	-	99.8	-	100.6
Fruit	-	8.3	71.1	-	-	9.5	-	8.3

the same conditions as that of the extracts. Identification of the phenolic compounds was done by comparing the BPC (base peak chromatogram) of standard phenolics from different extracts, Figure 2 (leaf extract) and Figure 3 (fruit extract) of the aerial part of *P. sarmentosum* resolved by the HPLC system. Gallic acid, tannic acid, quercetin, and naringin were the phenolic compounds found in both leaf and fruit of *P. sarmentosum* (Table 1). These four compounds were chosen based on the literature review on their antibacterial activity against plant pathogens (Wink, 1988; Zaidi–Yahiaoui *et al.*, 2008; Daglia, 2012) . The phenolic compound with the highest value is quercetin in the leaf of *P. sarmentosum* (100.6 µg/g dried weight (DW)), as compared to the fruit that contained only 8.3 µg/g dried weight (DW). The amounts of active compounds are different between parts of plants, hence the difference in the phenolic compound content between the leaf and fruit of *P. sarmentosum* as observed in this study. This was also observed in the concentration of diterpene lactones in *Andrographis paniculata* where the leaves contain a higher concentration of diterpene lactones (Kumar *et al*., 2004) while the seed contains the lowest concentration (Sharma *et al.*, 1992).

The quercetin content in the leaf extract (100.6 µg/g DW) was significantly higher ($P < 0.05$) among the phenolic compounds detected. This content is higher than

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the ones observed by Kuti and Konuru (2004) in raw *Cnidoscolusa conitifolius* (16.9 µg/g) and *Cnidoscolus chayamansa* (44.7 µg/g), whilst Crozier *et al*. (1997) observed a lower quercetin content in onion (201 µg/mg DW) and garlic (227 µg/g DW). This finding is supported by Miean and Mohamed (2001) who screened and detected quercetin in *P. sarmentosum* crude extract with a content of 30.5 mg/kg DW. The naringin content in the leaf (99.8 µg/g DW) is higher compared to the content in the skin (517.2 µg/g), juice (98.4 µg/g) and seed (29.2 µg/g) of rough lime. However, it is overall lower than the amount detected in the skin of pomelo; which contained a higher amount of naringin (3910 µg/g fresh weight) than the juice (220.0 µg/g fresh weight) (Yusof *et al*., 1990). Table 1 presents the concentration of phenolic compounds in the leaf and fruit of *P. sarmentosum*. The concentration of tannic acid with the values of 43.1 and 71.1 µg/g DW respectively is contrary from the findings reported by Graham (1992) who found no tannic acid in tea. The gallic acid content in the leaf (22.5 µg/g DW) was found to be higher than in the fruit (8.3 µg/g DW).

Standard calibration curve of the phenolic compounds

As shown in the results, the procedure and instrument parameters used for separating the phenolic chromatograms were suitable and accurate. As presented in Figure 4, gallic acid, tannic acid, quercetin, and naringin showed a linear response for 6 working standard solutions at concentrations of 10, 50, 100, 250, 500, and 1000 ppm. A linearity of calibration was assessed from the linear regression of response (area) versus concentration of phenolics. The lowest calibration level (LCL), i.e. the lowest level of calibration standard which run on an instrument with acceptable response, is 10 ppm.

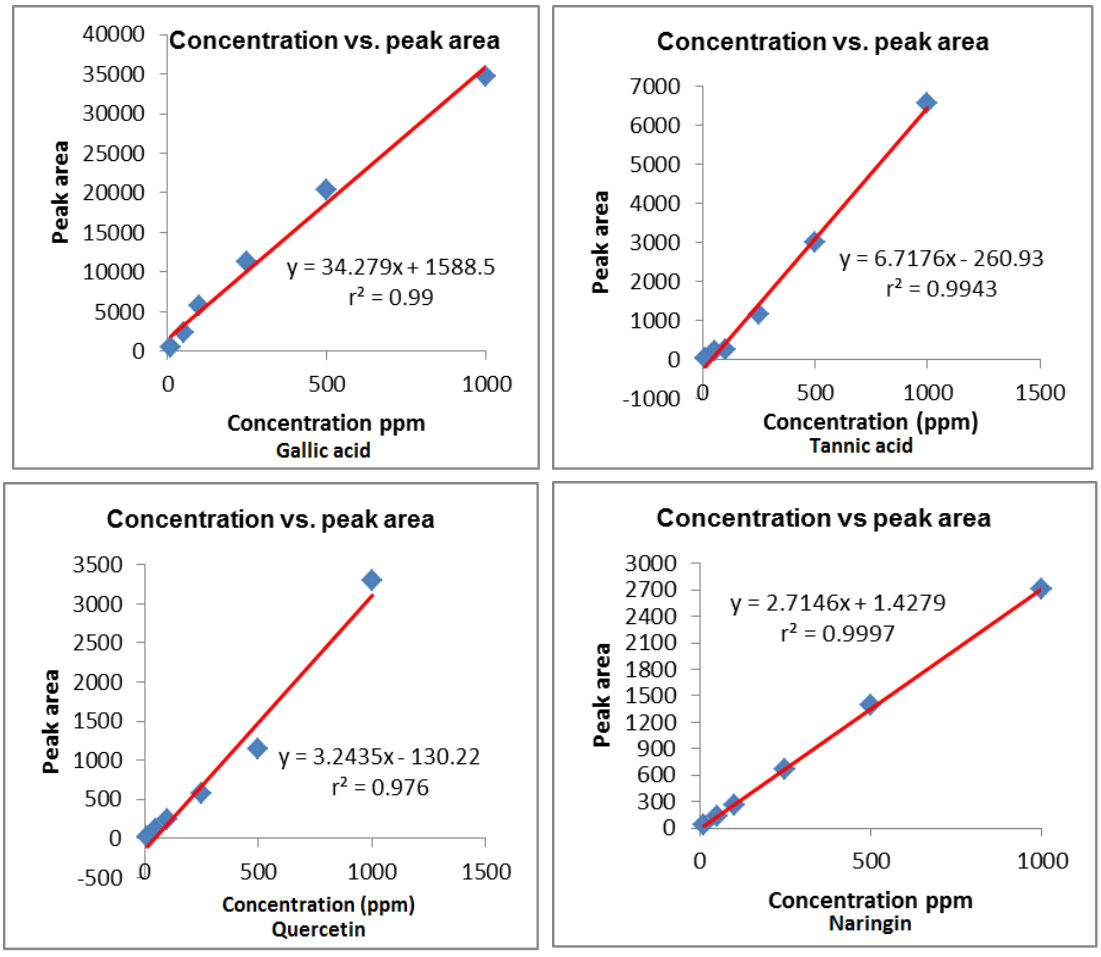

Figure 4: Linear calibration curve for gallic acid, tannic acid, quercetin and naringin.

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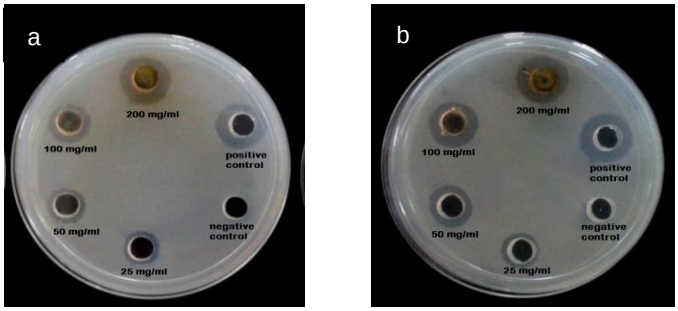

Figure 5: Inhibitory effects of the methanolic leaf extracts against (a) *P. fuscovaginae and* (b) *X. oryzae*.

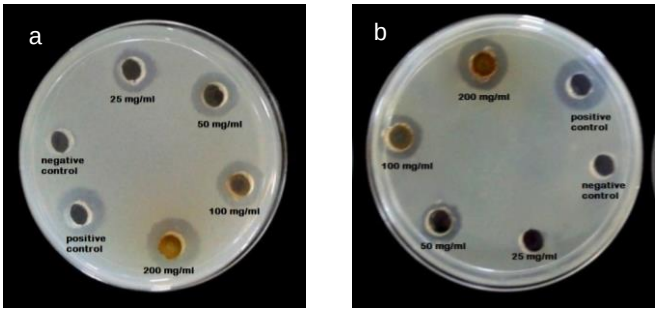

Figure 6: Inhibitory effects of the methanolic fruit extracts against (a) *P. fuscovaginae* and (b) *X. oryzae*.

Table 2: Antibacterial activity of *P. sarmentosum* crude extracts by agar well diffusion assay.

Microorganisms	Inhibition zone (cm)*			
	Leaf	Fruit	Streptomycin sulfate	Negative control
P. fuscovaginae	17.00±2.00	12.00±1.00	18.00±1.73	0
X. oryzae	15.33±0.58	17.33±0.58	21.00±3.46	0

Inhibition zone diameter ± SD (cm); analyses were done in triplicate

Table 3: Antibacterial activity of *P. sarmentosum* crude extracts by disc diffusion assay.

Microorganisms	Inhibition zone (cm)*			
	Leaf	Fruit	Streptomycin sulfate	Negative control
P. fuscovaginae	15.67±1.15	10.67±0.58	15.67±5.13	0
X. oryzae	13.67±0.58	16.33±0.58	17.67±0.58	0

Inhibition zone diameter ± SD (cm); analyses were done in triplicate

Table 4 Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *P. sarmentosum* methanol leaf and fruit extract against rice pathogenic bacteria by macro-broth dilution method.

Extracts	Tested bacteria	MIC (mg/mL)	MBC (mg/mL)
Leaf	*P. fuscovaginae*	12.5	25
	X. oryzae pv.oryzae	12.5	12.5
Fruit	*P. fuscovaginae*	12.5	25
	X. oryzae pv.oryzae	12.5	25

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Antibacterial activity

Tables 2 and 3 present the antibacterial assay against the Gram-negative rice pathogenic bacteria by agar well and disc diffusion assay, respectively. The concentration of the extracts was 100 mg/mL. From the results, In Figure 5, the leaf extract of *P. sarmentosum* showed a higher inhibition zone diameter against *P. fuscovaginae* while the fruit extract (Figure 6) showed a higher inhibition zone diameter against *X. oryzae* in both agar well and disc diffusion assay, respectively.

Generally, both of the extracts exhibited moderate to strong inhibitory activities against the *P. fuscovaginae* and *X. oryzae pv. oryzae* tested, which indicated the strong effects of the antibacterial agent that could access the bacterial cell that has long been known to possess an outer-membrane permeability barrier.

Table 4 presents the MIC and MBC of the methanolic extract of leaves of *P. sarmentosum* towards the tested bacterial strains by using broth macro dilution method. The *P. sarmentosum* leaf extract possessed bactericidal effects towards both bacterial strains tested. Between the two bacterial isolates, *X. oryzae* appeared to be the most susceptible to the extract. The lowest concentration of methanol extract that was able to inhibit the rice pathogenic bacteria was recorded at the concentration of 12.5 mg/mL.

Table 2 showed that the extract of *P. sarmentosum* leaf exhibited inhibitory activity against tested bacteria (inhibition zone diameter range: 15.33 - 17.00 cm) at 100 mg/mL. The bacteria reacted differently towards the extracts of leaf and fruit of the *P. sarmentosum* plant respectively. The leaf extract of *P. sarmentosum* showed high inhibition on *P. fuscovaginae* but exhibited low inhibition on *X. oryzae* compared to the fruit extract of *P. sarmentosum,* which showed high inhibition on *X. oryzae* but low inhibition on *P. fuscovaginae*. This is consistent with the findings of Cloete (2003), which explained that different bacteria will react differently to bactericides, either due to inherent differences such as unique cell envelope compositions and non-susceptible proteins or the development of resistance either by adaptation or genetic exchange. This reaction could also be related to the beneficial effects of phytochemicals through the additive or synergistic actions of several bioactive compounds (which acts) at single or multiple target sites (Tyler *et al*., 1999). However, the inhibition of the extracts was low compared to Streptomycin (30 µg/well), which is not surprising. Higher activities could also be expected if isolated compounds with antibacterial activity from the extracts had been employed in the assay. These results might also be related to the presence of active phytochemicals in the leaf such as 1-allyl-2,6-dimethoxy-3,4-methylenedioxybenzene, 1-allyl-2,4,5-trimethoxy benzene, 1-(1-E-propenyl)-2,4,5-trimethoxybenzene, and 1-allyl-2-methoxy-4,5-methylenedioxybenzene. These compounds showed antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* (Masuda *et al.*, 1991). *Piper sarmentosum* has also been found to have strong antifeedant and toxicity effects on *Brontispa longissima,* a destructive pest to palm plants (Qin *et al*., 2010).

As reported in a previous study by Fernandez *et al*. (2012), the antibacterial activities observed in the leaf and fruit of *P. sarmentosum* may be attributed to the presence of some secondary metabolites in the plant extract. Lattanzio *et al*., (2006) and Maddox *et al*. (2010) also described that the phenolic and flavonoids present in the extracts from various medicinal plants possess antimicrobial activity. A study by Vaquero *et al.* (2007) also reported on the activities of different wine containing gallic, caffeic, vanillic acid, rutin, and quercetin against pathogenic microorganisms. From the study, *Escherichia coli* exhibited as the most sensitive bacterium and in contrast, *Flavobacterium* sp. was found resistant against all phenolic compounds tested. The phenolic and flavonoids analyses showed the presence of tannic acid, gallic acid and quercetin in leaf and fruit of *P. sarmentosum.*

The mechanisms of action of these compounds might contribute to the antibacterial activity of *P. sarmentosum* leaf and fruit. This is in line with the findings of Cushnie and Lamb (2005) who reported on the antimicrobial activity of naringin, quercetin, and rutin against human pathogenic microbes with mechanisms of action such as energy metabolisms, cytoplasmic membrane function, and nucleic acid synthesis.

The presence of Gallic acid in the leaf and fruit of *P. sarmentosum* might be one of the reasons for the antibacterial activity of the extract against the tested bacteria. This is in line with the observation by Chanwitheesuk *et al.* (2007), who reported the potent activity of Gallic acid against eight human pathogenic bacteria and six fungal strains. Gallic acid has been known to exhibit some pharmacological activities (Inoue *et al*., 1995). This is supported by a study conducted by Kawada *et al.* (2001) which described that a naturally occurring plant phenol, gallic acid (3,4,5-trihydroxybenzoic acid), can induce apoptosis in four different types of human lung cancer cell lines *in vitro*. It was suggested that the combination of this gallic acid and an anti-cancer drug may be an effective alternative for treatment of lung cancer.

The presence of naringin in the leaf and fruit of *P. sarmentosum* could be supported by the inhibition on the growth of tested bacteria such as in a previous study that showed naringin to be effective against Gram-positive than Gram-negative bacteria (Negi and Jayaprakasha, 2001; Celiz *et al*., 2011). In addition, a study conducted by Li and Xu (2008) and Reagor *et al.* (2002) stated that naringin and quercetin exhibit antimicrobial activity.

The antimicrobial action of phenolic compounds has been reported to have a relation with the inactivation of cellular enzymes, which generally depends on the penetration rate of the compounds into the cell or it could also be triggered by changes in membrane permeability (Cowan, 1999). Membrane permeability is the major key in the mechanism for plant antimicrobial action. The compounds disrupt the pathogenic cell membrane and causes a loss of cellular integrity and eventual cell death

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(Lewis and Ausubel, 2006).

CONCLUSION

As a conclusion, this study proved that *P. sarmentosum* leaf and fruit consists of gallic acid and tannic acid as the phenolic acid components; and quercetin and naringin as the flavonoid components. Furthermore, extracts of the leaf and fruit from this plant are found to be potent and could serve as effective antibacterial agents. With regards to this study, we can conclude that the leaf and fruit extract from this plant can be introduced further as alternatives of the current control options with low cost, non-toxic, and better effectiveness at a lower concentration. Subsequently, it can be further developed as a novel bactericide. In the future, field experiments should be carried out to evaluate whether the extracts from *P. sarmentosum* can be used to control bacterial leaf blight and sheath brown rot of rice, and possibly other bacterial diseases of other crops under practical farming conditions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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