

## Bioprotective properties of three Malaysia *Phyllanthus* species: An investigation of the antioxidant and antimicrobial activities

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**Abstract:** The aim of this work was to investigate the antioxidant and antimicrobial of *Phyllanthus amarus*, *Phyllanthus niruri* and *Phyllanthus urinaria*. *P. niruri* was found to possess the highest antioxidant activity, the activity decreased in the order *P. niruri* > *P. amarus* > *P. urinaria* for water extract. However, the activity decreased in the order *P. niruri* > *P. urinaria* > *P. amarus* for methanol extract. The result correlation between the antioxidant activity and total phenolic content revealed a positive correlation of  $0.954 < r^2 < 1.000$  for both water and methanol extract. Methanol extract showed higher total phenolic content and antioxidant activity as compared with water extract. Lowest Minimum Inhibitory Concentration (MIC) value for water extract against the selected microorganism was >2.5 mg/mL meanwhile, for methanol extract was <0.625 mg/mL. MBC level >2.5 mg/mL and >0.625 mg/mL were the value for water and methanol extract. Methanol extract showed better inhibition potential than water extract.

**Keywords:** Antioxidant, antimicrobial, DPPH free radical scavenging assay, ferric reducing antioxidant potential

### Introduction

Reactive oxygen species (ROS) such as superoxide radical anion, hydroperoxyl radical are generated in cells from byproducts of metabolism and responsible for the development of a wide number of degenerative diseases such as cardiovascular disease (CVD), diabetic, cirrhosis and several cancers (Halliwell, 1996). All organisms have their own antioxidant defense system to protect them against free radical damage by enzymes, such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherol and glutathione (Mau *et al.*, 2002). However, our body system sometimes cannot work efficiently that lead to unavoidable damage caused by oxidation. As a result, consumption of additional antioxidants through herbs, foods or supplements (phytochemicals) are beneficial to defense against the harmful ROS (Shi *et al.*, 2001). A recent investigation on the beneficial effects of fruits, vegetables and spices containing high phytochemical antioxidants were carried out by groups of researchers (Aziz *et al.*, 2007; Lim *et al.*, 2007) concluded that the crude extracts with antimicrobial and antioxidant properties are bioprotective in nature and are therefore valuable in the food industry for longer shelf life. Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT) and Tert-butylated hydroxyquinone (TBHQ)

are the common synthetic antioxidants to prevent oxidative process (Lörliger, 1991) that have been used in food industry since 1940 but the trend currently is to move towards natural antioxidants as an alternative source (Kartal *et al.*, 2007). Botterweck *et al.* (2000) revealed that BHA and BHT were found to promote tumor formation, therefore the use of a natural antioxidant source become crucial.

Phytochemicals that produced by the plant kingdom for defense, protection, cell to cell signaling and as attractants for pollinators (Kartal *et al.*, 2007) are bioactive non-nutrient plant compounds that have been linked to the reduction of risk related to chronic diseases. Their commercial potential can be utilized in food industry as additives, as antibiotics in pharmaceutical and as essence extract in herbal and cosmetic products. Phytochemicals are structurally diverse, based on their biosynthetic origins they can be classified into basically four classes whose members may exert positive effects on human health; the terpenoids, phenolics and polyphenolics and nitrogen-containing alkaloids and sulphur-containing compounds (Crozier *et al.*, 2006).

Recent studies conducted globally have indicated that food borne-diseases are increasing, for example in United States account for 76 million illnesses and five thousand of death every year (Mead *et al.*, 1999), presence of *Listeria monocytogenes* in food

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(Jeyaletchumi *et al.*, 2010), indication of various *Salmonella* in vegetables (Learn Han *et al.*, 2009) and occurrences of *Campylobacter* in chicken (Usha *et al.*, 2010). Therefore, any research elucidating antibacterial and antioxidant properties of plant extract could prove to be a promising alternative to those antibiotics, which are becoming ineffectual against bacterial infections (Goel, 2008).

Malaysia's rainforest being part of the world's tropical rainforest is also considered as one of the most evolved and diverse rainforest in the world. This biodiversity supports numerous species of medicinal plants. One of such genus that have been used for in folk medicine for decades and known to possess great diversity of secondary metabolites is the genus *Phyllanthus*. *P. niruri*, *P. urinaria*, *P. amarus* also locally known as "dukung anak" (Masturah *et al.*, 2006) has been use traditionally used for treating kidney and gallbladder stones, liver related diseases and viral infection. Research on *Phyllanthus* sp. has been widely conducted in India. (Harish and Shivanandappa, 2004) reported that antioxidant activity and hepatoprotective potential found in *P. niruri*. Meanwhile, *P. amarus* was reported to have anti-diabetic, anti-cancer and anti-inflammation properties and was shown to have anti-mutagenic and anti-carcinogenic effects in India (Sripanidkulchai *et al.*, 2002). In addition, antioxidant activities from methanol extract of five *Phyllanthus* species in India have been reported by Kumaran and Karunankaran (2005).

Although there has been some reports on the health benefits of *Phyllanthus* sp elsewhere but information regarding the antioxidant and antibacterial activities of these three species in Malaysia is very limited. This study was therefore undertaken to compare the three *Phyllanthus* sp in terms of its potential antioxidant and antimicrobial activity. Additionally, two types of extraction method were used in this study and their efficiencies in extracting the beneficial phytochemical were compared through antioxidant and antibacterial assays. Lastly, a correlation between the total phenolic content and total antioxidant activity was determined. The antioxidant and antibacterial comparison in this study are the first report in Malaysia.

## Materials and Methods

### Plant material

All the *Phyllanthus* sp used in this study were obtained from the collection of plants grown in Malaysia Agricultural Research and Development Institute (MARDI) which were taxonomically identified by a qualified botanist. To minimize variation

of effects of environmental and growth conditions on the growth and production of phytochemicals in the plants under this study, all the plants were grown under semi-controlled conditions in a nethouse. They were subjected to similar applications of levels of fertilizers, soil media and irrigation.

### Extraction

Whole plants except root were harvested, rinsed and air-dried for few minutes at room temperature. The plant material were cut into smaller pieces and ground with liquid nitrogen prior to freeze-drying. 1g of freeze-dried powder was extracted with 40 mL of water extracting buffer (20 mM diethyldithiocarbamic acid, 0.5% formic acid) and mixed thoroughly for 30 minutes. The extract was then centrifuged at 8900 rpm, 4°C for 5 mins and filtered with WHATMAN No. 40. The residue was resuspended in 20 mL of water and the same extraction protocol was repeated twice to obtain a total of 80 mL of crude extract which was then subjected to the antioxidant activity assay. Same extraction protocol was applied for methanol extraction.

### Determination of total phenolic content (TPC)

A slight modification of the total phenolic content assay was adopted from previous study (Lim *et al.*, 2007). A standard calibration curve was plotted using different concentrations of gallic acid and the absorbance was recorded at 765 nm. 0.3 mL of aliquot extract (with appropriate dilution, if necessary) was mixed with 1.5 mL Folin-Ciocalteu (previously diluted 10x with water) followed by 1.2 mL sodium carbonate. The mixture was then vortexed thoroughly and kept in dark at room temperature for 30 minutes. Subsequently, the absorbance was measured at 765 nm using Perkin Elmer Lambda 25 UV/ VIS Spectrophotometer. 3-hydroxyphenylacetic acid (100 µg/mL) was used as positive control and the water extracting buffer as the negative control. Results were calculated as mg gallic acid equivalent per g dry weight plant (mg GAE/ g dry weight plant). All assays were carried out in triplicates.

### Analysis of antioxidant activity assay

#### 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay

A slight modification of the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) antioxidant assay was adopted from Wong, Leong and Koh (2006). DPPH is one of the few stable and commercially available organic nitrogen radicals (Dejian *et al.*, 2005) exhibiting a dark purple color at absorbance 515 nm. In the process when free

radicals are being scavenged, DPPH will be reduced, producing a yellow coloration. 3 mL of DPPH (0.1 mM) was added to 0.1 mL sample (with appropriate dilution, if necessary) and mixed thoroughly. It was left in dark for 30 minutes at room temperature. Ascorbic acid (10 µg/mL) was used as a positive control and appropriate extracting buffer was used as a negative control. Absorbance was measured at 515 nm after incubation. A standard calibration curve using different concentrations of Trolox was plotted. Results were expressed in mg Trolox equivalent per g dry weight plant (mg TE/ g dry weight plant). All analysis was carried out in triplicates.

#### *Ferric reducing antioxidant potential (FRAP) assay*

Ferric Ion reducing ability (FRAP) assay, described by Jaganath (Jaganath, 2005) was used to estimate the antioxidant capacity of the three *Phyllanthus* species. This method measures the ability of a solution to reduce a ferric-tripyridyl-triazine complex ( $\text{Fe}^{3+}$ -TPTZ) to the ferrous form,  $\text{Fe}^{2+}$  producing a blue color with the absorption at 593 nm. FRAP reagent was prepared freshly with a ratio of 1:1:10, 1 part of 10 mM TPTZ mixed with 1 part of 20 mM ferric chloride and 10 parts of acetate buffer at pH 3.6. An aliquot measuring 0.1 mL of extract (with appropriate dilution, if necessary) was dissolved with 0.3 mL water followed by 3 mL of FRAP reagent. After 4 minutes of incubation in dark, the absorbance was measured at 593 nm and compared to a 0.0625 to 4.000 mM  $\text{Fe}^{2+}$  standard calibration curve. Quercetin hydrate (10 µg/mL) was used as a positive control and appropriate extracting buffer was used as a negative control. Results were expressed in mM  $\text{FeSO}_4$ /g dry weight plant. All analysis was done in triplicates.

#### **Bacterial isolate and growth condition**

The potential antibacterial activities of all extracts were analyzed against the following bacteria: *Salmonella typhimurium* (ATCC 14028), *Serratia marcescens*, *Listeria monocytogenes*, *Staphylococcus aureus* (ATCC33591), *Salmonella paratyphi A* (ATCC 9150), *Klebsiella oxytoca*, *Aeromonas hydrophila*, *Escherichia coli*, *Yersinia enterocolitica*, *Bacillus niacini*, *Bacillus cereus*, *Bacillus anthracis*, *Bacillus arvi*, and *Methylobacterium hispanicum*. All strains were obtained from the collection ATCC, food and environmental isolates of Department of Biomedical Science, University Putra Malaysia. All bacterial isolates were maintained in nutrient agar plate and incubated at 37°C for 18 hours. For antimicrobial activity determination, single colony was selected from fresh culture from all strains and grown in 5 mL nutrient broth for 18 hours at 37°C.

#### **Antibacterial preliminary screening**

The antibacterial preliminary screening was performed using well diffusion assay. All strains were freshly cultured in nutrient agar plate before inoculating into a nutrient broth. A total of 100 µL of culture suspension ( $10^8$  CFU/mL) for each strain was spread evenly on the Mueller-Hinton agar. A well with the estimated size of 0.7 cm was made on the agar plate with appropriate agar thickness. 50 µL of water extract with 100 mg/mL and methanol extract with 50 mg/mL were pipetted into the well. Gentamicin (10 µg) was used as a positive control and appropriate solvents such as water and DMSO were used as a negative control. Plates were incubated overnight at 37°C prior to measuring the diameter of the inhibition zone for each tested extracts. All analysis was carried out in triplicates. Strains that exhibit a positive response to the extracts were selected for the subsequent analysis.

#### **Minimum inhibition concentration (MIC) and minimum bactericidal concentration determination (MBC)**

A slight modification of method from MIC and MBC protocols adopted from Kukić *et al.* (2008) was used. To carry out this analysis, the crude water extract was dissolved in water while DMSO was used as the solvent for the crude methanol extract. Serial dilution of the plant extract was carried out, using broth as the diluents prior to addition of the inoculum. The final inoculum concentration was  $10^7$  CFU/mL while the final concentration of water extract ranged from 2.5 mg/mL to 20 mg/mL and for methanol extract the concentration ranged from 0.625 mg/mL to 10 mg/mL. The mixture was then incubated overnight at 37°C. The optical density absorbance was measured at 600 nm. Water and DMSO were applied as negative controls and the gentamicin was applied as a positive control. The lowest concentration with no visible growth and inhibition activity more than 90% ( $\text{IC}_{90}$ ) by absorbance calculation was defined as MIC.

Meanwhile, 5 µL containing mixture of inoculums and extract from overnight reactions were plated on nutrient agar and further incubated at 37°C in order to investigate the minimum bactericidal concentration. The lowest concentration with no visible growth on nutrient agar plate was determined as MBC. Inoculum dilution was adjusted to the standardized concentration and cultured on the agar plate in order to check the absence of contamination and cell viability. All analysis was repeated in triplicates.



## Results and Discussions

### Total phenolic content (TPC) determination and total antioxidant activities analysis

The TPC assay is a common assay widely used to estimate relative amounts of phenolic compounds present in an extract. The TPC results were expressed as mg gallic acid equivalent as this compound represents the most simple form of a phenolic compound. Phenolic compounds present in the extract undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the TPC reagent (Wong *et al.*, 2006). Depending on the number of phenolic groups present, different response can be observed in terms of the color change due to oxidation of the TPC reagent. This color change is detected by a spectrophotometer and quantified in term of mg gallic acid equivalent per dry weight plant.

The two antioxidant assays that have been used in the study were DPPH scavenging assay and FRAP antioxidant assay. 1,1-Diphenyl-2-picrylhydrazyl is the main chemical used in the DPPH assay and it is used widely for the determination of free radical scavenging activity of antioxidant compounds in extracts (Aziz *et al.*, 2007). It is also one of the few known stable and commercially available organic nitrogen free radical (Dejian *et al.*, 2005). When this compound is added to the plant extracts containing antioxidant compounds, diphenylpicrylhydrazyl is reduced to diphenylpicrylhydrazine and a color change is observed in the process where the color fades from purple to yellow. This then can be measured using UV-VIS spectrophotometer at absorption 515 nm. The antioxidant activity can be expressed in various ways and one of the most common ways is to express by referring it to a common reference standard. One common reference standard used for this purpose is (S)-(-)-6 hydroxy-2,5,7,8-teteramethylchroman-2-carboxylic acid, also known as Trolox. Therefore, all the antioxidant activities for DPPH assay was expressed as mg Trolox equivalent per g dry weight of plant. The ferric ion reducing antioxidant power (FRAP) assay is based on the electron transfer mechanism. Antioxidant compounds from the plant extract are capable of donating single electron to the

ferric-TPTZ complex which is the main component in the assay. This reaction will cause a reduction of  $Fe^{3+}$  -TPTZ into blue ferrous-TPTZ complex, with absorption at 593 nm.

The results for the TPC analysis indicate that all *Phyllanthus* sp. contain significant amounts of phenolic compounds (Table 1) as revealed by the concentrations when compared to the positive control. When comparing the three *Phyllanthus* species, quantity of phenolics in the water extract in decreasing order was *P. niruri* > *P. amarus* > *P. urinaria*. However, this order differed slightly for methanol extract, which is *P. niruri* > *P. urinaria* > *P. amarus*. The same patterns of ranking were also observed for the antioxidant DPPH and FRAP assays. Correlation analysis was carried out between TPC and DPPH and also between TPC and FRAP. Result confirmed a convincing correlation of  $R^2$  value of 0.95 to 1.0 between phenolic content and antioxidant activity (Table 2). These result suggested that over 95% of the antioxidant capacity in *Phyllanthus* was due to the contribution of phenolic compounds. The other 5% may be due to other non phenolics compounds that exhibit antioxidant properties

Overall it can be observed from Table 1 that both the total phenolic content and antioxidant activity was consistently higher in the methanol extract when compared with the water extract. The probable reason for this is due to the difference in the polarity solvents used. The polarity index for water and methanol are 9.0 and 6.6. Methanol is more efficient in extracting wider range of phenolic compounds (from polar to semipolar) found in *Phyllanthus*. This research finding is supported by investigation carried out by Masturah *et al.* (2006) where the major components of *Phyllanthus* sp are active hydrolysable tannins that can be extracted using ethanol-water mixture which are semipolar compounds such as ellagitannins and gallotannins (Tian *et al.*, 2009). A significant difference of antioxidant potential among the three *Phyllanthus* species ( $p < 0.05$ ) was observed for both water and methanol crude extract. Among the three tested species, crude methanol and water extract of *P. niruri* scored the highest antioxidant potential activity followed by *P. urinaria* and *P. amarus* (Table 1). The finding was supported by Harish *et al.* (2004) that leaf

**Table 1.** Determination of total phenolic content and antioxidant activity analysis

Species	Total phenolic content (TPC) <sup>a</sup>		DPPH Scavenging Assay (DPPH) <sup>b</sup>		FRAP Antioxidant Assay (FRAP) <sup>c</sup>	
	water extract	methanol extract	water extract	methanol extract	water extract	methanol extract
<i>Phyllanthus amarus</i>	46.71 ± 0.514	50.24 ± 2.158	364.46 ± 7.802	472.06 ± 17.294	626.16 ± 31.252	542.93 ± 19.305
<i>Phyllanthus niruri</i>	55.38 ± 0.496	68.70 ± 2.869	443.47 ± 7.229	686.04 ± 19.156	731.65 ± 28.963	936.48 ± 20.657
<i>Phyllanthus urinaria</i>	41.19 ± 0.689	62.56 ± 4.097	309.98 ± 12.330	652.19 ± 20.502	426.56 ± 12.172	742.19 ± 15.375
Positive Control	56.05 ± 0.245		439.97 ± 15.550		266.21 ± 18.339	

<sup>a</sup> express as mg GAE/g dry weight, 100 µg/mL of 3-hydroxyphenylacetic acid as positive control

<sup>b</sup> express as mg TE/g dry weight, 10 µg/mL of ascorbic acid as positive control

<sup>c</sup> express as mM FeSO<sub>4</sub> equivalent/ g dry weight, 10 µg/mL of quercetin hydrate as positive control

and fruit extracts from *P. niruri* exhibited antioxidant activity.

A strong correlation between total phenolic content from both water and methanol extract with both DPPH scavenging assay and FRAP assay ( $r^2$ ) ranged from 0.954 to 1.00. Thus, total phenolic content increased proportional with the antioxidant activity. The satisfactory correlation reflected that polyphenol in the extracts played an important role in antioxidant activity and best supported by report from Teissedre & Landrault (2000) which phenolic compounds have been discovered possess antioxidant activity in the inhibition of LDL oxidation. It can be a good antioxidant agent among others category of compounds. Only the correlation between the total phenolic content and DPPH scavenging assay of water extract showed significant level ( $p=0.014$ ,  $<0.05$ ). Others correlations were at mild significant level (Table 2). Indication from mild level of satisfactory happened due to the total phenolic content is not a specific test for polyphenol compounds and the antioxidant activity may due to more than 1 phenolic group to react. Moreover, others group of secondary metabolite such as flavanoid may contribute to antioxidant activity as well beside phenolic compounds. This has been proven from the percentage increased of total phenolic content from water extract to methanol extract is not as high as the percentage increased of total antioxidant activity tremendously from water to methanol extract which consist of almost 50% increased. Thus, they might be other compounds play the role as antioxidant during assay.

**Table 2.** Correlation between total phenolic content and antioxidant activity

Total phenolic content	Correlation, $r^2$			
	DPPH	p	FRAP	p
Water extract	1.000	0.014	0.954	0.193
Methanol extract	0.983	0.118	0.983	0.117

#### Antibacterial activity determination

Preliminary screening result (table 4) showed that both methanol and water extract possesses limited spectrum of antimicrobial activity. There was no inhibition effect against bacteria gram negative among the selected pathogen. However, the inhibition zone for 100 mg/mL water extract against gram positive bacteria ranged from 1.15 to 1.65 cm in diameter. The inhibition zone which showed from 50 mg/mL methanol extract ranged between 1.40 cm and 1.70 cm in diameter. As overall, methanol extract showed better antibacterial inhibition effect at lower concentration as compared with water extract. All the tested gram positive bacterial have been inhibited except *B. arvi* and *B. niacini*.

#### MIC and MBC antimicrobial determination

MIC value for water extract of all species against the selected gram positive bacterial was  $> 2.5$  mg/mL except for *P. niruri* water extract which against *L. monoxytogenes* was  $< 2.5$  mg/mL. Majority MBC values of water extracts were  $> 2.5$  mg/mL. As a conclusion, water extract of *P. niruri* was able to inhibit the *L. monoxytogenes* at the lowest concentration of  $< 2.5$  mg/mL. MIC value of methanol extract from 3 species showed  $> 0.625$  mg/mL against *B. anthracis* and *B. cereus*. However, the MIC value against *L. monoxytogenes*, *M. hispanicum* and *S. aureus* was the lowest value,  $< 0.625$  mg/mL. The lowest MBC value was  $> 0.0625$  mg/mL for *P. amarus* and *P. niruri* against *L. monoxytogenes*. Overall, methanol extract have higher potential inhibiting and killing properties towards the gram positive bacterial based on the MIC and MBC value.

Based on the MIC and MBC results, it was clearly seen that methanol extract consists of higher content of potential antimicrobial compounds which is useful as new antibiotics. The antimicrobial activity from methanol was as strong as water extract at 2 fold lower the concentration at preliminary screening and 3 fold lower of MIC value. From the result obtained, methanol extract of *P. niruri* and *P. amarus* are the potential product against *L. monoxytogenes*. Beside that, both local herbs are potential to be new antioxidant and antibacterial agent. *L. monoxytogenes* causes listeriosis is frequently isolated from a wide variety of foods and dairy products. It is capable to grow at refrigerator temperature and increase the numbers during food's shelf life (Tortora *et al.*, 2001). As a conclusion, *P. niruri* and *P. amrus* can be the newly discovered natural product as antioxidant which is useful for human and as antibacterial in food industry.

There are various types of inhibition mechanism by the commercial antibiotics, such as, inhibit DNA replication, inhibit protein synthesis, lysis of cell membrane, inhibition of ion exchange function and dysfunction on signaling transduction. The antibacterial result from this study was clearly seen that only inhibited gram positive instead of gram negative. The significant difference between gram positive and gram negative is the existing of a peptidoglycan layer of cell wall for gram positive bacteria and the unique outer membrane for gram negative bacteria. The outer membrane of the gram negative cell consists of lipopolysaccharides, lipoproteins and phospholipids as a major protection. It provides a barrier to certain antibiotics, digestive enzyme, detergent and heavy metal (Tortora *et al.*, 2001). Peptidoglycan layer consists of repeating

**Table 3.** Diameter of inhibition zone result from preliminary screening against selected bacterial (cm ± SD)

Bacterial strains	Gram	water extract (100mg/mL)			methanol extract (50mg/mL)		
		1	2	3	1	2	3
<i>Salmonella typhimurium</i>	Negative	-	-	-	-	-	-
<i>Klebsiella oxytoca</i>	Negative	-	-	-	-	-	-
<i>Aeromonas hydrophila</i>	Negative	-	-	-	-	-	-
<i>Escherichia coli</i>	Negative	-	-	-	-	-	-
<i>Salmonella paratyphiA</i>	Negative	-	-	-	-	-	-
<i>Yersenia enterocolitica</i>	Negative	-	-	-	-	-	-
<i>Serratia marcescens</i>	Negative	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	Positive	1.60 ± 0.000	1.65 ± 0.071	1.65 ± 0.071	1.70 ± 0.283	1.65 ± 0.212	1.70 ± 0.000
<i>Listeria monocytogenes</i>	Positive	1.35 ± 0.212	1.50 ± 0.141	1.40 ± 0.000	1.70 ± 0.000	1.50 ± 0.000	1.65 ± 0.071
<i>Bacillus niacini</i>	Positive	-	-	-	-	-	-
<i>Bacillus cereus</i>	Positive	1.30 ± 0.141	1.30 ± 0.141	1.25 ± 0.071	1.55 ± 0.212	1.50 ± 0.141	1.60 ± 0.141
<i>Bacillus anthracis</i>	Positive	1.15 ± 0.071	1.25 ± 0.071	1.20 ± 0.141	1.55 ± 0.071	1.40 ± 0.141	1.55 ± 0.071
<i>Bacillus arvi</i>	Positive	-	-	-	-	-	-
<i>Methylobacterium hispanicum</i>	Positive	1.35 ± 0.071	1.30 ± 0.000	1.20 ± 0.000	1.60 ± 0.141	1.50 ± 0.000	1.60 ± 0.141
Gentamicin (10µg)					1.95 ± 0.055		
Water / DMSO					Negative		

1- *P. amarus* crude extract, 2- *P. niruri* extract, 3- *P. urinaria* crude extract

**Table 4.** Minimum inhibition concentration (MIC, > IC<sub>90</sub>) and minimum bactericidal concentration (MBC) of crude extract against selected pathogen

Pathogens	Water crude extract						Methanol crude extract					
	MIC (mg/mL)			MBC (mg/mL)			MIC (mg/mL)			MBC (mg/mL)		
	1	2	3	1	2	3	1	2	3	1	2	3
<i>Bacillus anthracis</i>	5.0	5.0	5.0	5.0	5.0	10.0	1.25	1.25	1.25	2.5	>10	2.5
<i>Bacillus cereus</i>	5.0	5.0	5.0	5.0	5.0	10.0	1.25	1.25	1.25	2.5	2.5	2.5
<i>Listeria monocytogenes</i>	5.0	≤2.5	5.0	5.0	5.0	5.0	≤0.625	≤0.625	≤0.625	1.25	1.25	2.5
<i>Methylobacterium hispanicum</i>	5.0	5.0	5.0	5.0	10.0	20.0	≤0.625	≤0.625	≤0.625	5.0	2.5	5.0
<i>Staphylococcus aureus</i>	5.0	5.0	5.0	10.0	10.0	10.0	≤0.625	≤0.625	≤0.625	2.5	2.5	2.5

1- *Phyllanthus amarus*, 2- *Phyllanthus niruri*, 3- *Phyllanthus urinaria*  
 >IC<sub>90</sub> percent inhibition of Gentamicin (10 µg) as positive control  
 No growth in water and DMSO as negative control

disaccharide attached by polypeptides to protect cell. As a result, active gradients of both potential herbs may target to the peptidoglycan cell wall of the bacteria which indirectly resulted in the membrane layer easily being lysed. However, a further study on the actual mechanism is needed especially using Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM).

## Conclusions

In conclusion, results obtained from this study exhibited that both local herbs tested are potential to be new antioxidant and antibacterial agent especially *P. niruri*. As a conclusion, *P. niruri*, *P. amarus* and *P. urinaria* are considered as newly discovered natural product as antioxidant source which is useful for human consumption and possesses antibacterial properties targeting gram positive bacteria.

## Acknowledgements

The authors would like to thank Biotechnology

Research Centre, Malaysian Agricultural Research and Development Institute for the kind support in terms of scientific collaboration and the supply of the samples for this study.

## References

- Aziz, T, Mehmet, E. D., Nazime, M., Ibrahim, K. and Kudret, G. 2007. Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. Food Chemistry 101: 267-273.
- Botterweck, A. A. M., Verhagen, H., Goldbohm, R. A., Kleinjans, J. and Brandt, P. A. 2000. Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: result from analyses in the Netherlands cohort study. Food and Chemical Toxicology 38: 599-605.
- Crozier, A., Yokota, T., Jaganath, I. B., Mark, S. C., Saltmarsh, M. and Clifford, M. N. 2006. Secondary metabolites in fruits, vegetables, beverages and other plant-based dietary components. In Crozier, A., Ashihara, H. and Clifford, M. N. (eds.), Plant Secondary Metabolites and the Human Diet. Blackwells: Oxford, pp. 208-302.
- Dejian, H., Boxin, O. and Ronald, L. P. 2005. Review of

- The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry* 53: 1841-1856.
- Goel, S. 2008. Bioprotective properties of Turmeric: An investigation of the antioxidant and antimicrobial activities. *Journal of Young Investigator* 16: 12.
- Halliwell, B. 1996. Antioxidant in human health and disease. *Annual Review of Nutrition* 16: 33-50.
- Harish, R. and Shivanandappa, T. 2004. Antioxidant activity and hepatoprotective potential of *Phyllanthus niruri*. *Food Chemistry* 95: 180-185.
- Jaganath, I. B. 2005. Dietary Flavanoids: Bioavailability and biosynthesis. Phd thesis, University of Glasgow: UK.
- Jeyaletchumi, P., Tunung, R., Margaret, S. P., Son, R., Farinazleen, M. G. and Cheah, Y. K. 2010. Detection of *Listeria monocytogenes* in foods. *International Food Research Journal* 17: 1-11.
- Kartal, N., Sokmen, M., Tepe, B., Daferera, D., Polissiou, M. and Sokmen, A. 2007. Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure. *Food Chemistry* 100: 584-589.
- Kukić, J., Popović, V., Petrović, S., Mucaji, P., Cirić, A., Stojković, D. and Soković, M. 2008. Antioxidant and antimicrobial activity of *Cynara cardunculus* extracts. *Food Chemistry* 107: 861-868.
- Kumaran, A. and Karunakaran, R. J. 2005. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *Food Science and Technology* 40: 344-352.
- Learn-Han, L., Yoke-Kqueen, C., Shiran, M. S., Sabrina, S., Noor Zaleha, A. S., Sim, J. H., Chai-Hoon, K. and Son, R. 2009. Molecular characterization and antimicrobial resistance profiling of *Salmonella enterica* subsp. *enteric* isolated from 'Selom' (*Oenathe stolonifera*). *International Food Research Journal* 16: 191-202.
- Lim, Y. Y., Lim, T. T. and Tee, J. J. 2007. Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry* 103: 1003-1008.
- Löliger, J. 1991. The use of antioxidants in foods. Free radicals and food additives. London: Taylor & Francis.
- Masturah, M., Masitah, M., Wan Ramli, W. D., Harcharan, S. and Jamaliah, M. J. 2006. Extraction of hydrosable tannins from *Phyllanthus niruri* Linn : Effects of solvents and extraction method. *Separation and Purification Technology* 52: 487-498.
- Mau, J. L., Lin, H. C. and Song, S. F. 2002. Antioxidant properties of several specialty mushrooms. *Food Research International* 35: 519-526.
- Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M. and Tauxe, R. V. 1999. Food related illnesses and death in the United States. *Emerging Infectious Diseases* 5: 607-625.
- Shi, H. L., Noguchi, N. and Niki, E. 2001. Introducing natural antioxidants. In J. Pokorny *et al.*, Antioxidants in Food: practical applications, (eds.) Woodhead Publishing Ltd. and CRC Press.
- Sripanidkulchai, B., Tattawasart, U., Laupatarakasem, P., Vinitketkumneun, U., Sripanidkulchai, K., Furihata, C. and Matsushima, T. 2002. Antimutagenic and anticarcinogenic effects of *Phyllanthus amarus*. *Phytomedicine* 9: 26-32.
- Teissedre, P. L. and Landrault, N. 2000. Wine phenolics: Contribution to dietary intake & bioavailability. *Food Research International* 33: 461-467.
- Tian, F., Li, B., Ji, B. P., Yang, J. H., Zhang, G. Z., Chen, Y. and Luo, Y.C. 2009. Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*: The polarity effects the bioactivities. *Food Chemistry* 113: 173-179.
- Tortora, G. J., Funke, B. R. and Case, C. L. 2001. *Microbiology: An Introduction* (7<sup>th</sup> ed.). USA: Daryl Fox Publisher, pp86-89
- Usha, M. R., Fauziah, M., Tunung, R., Chai, L. C., Cheah, Y. K., Farinazleen, M. G. and Son, R. 2010. Occurrence and antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* in retail broiler chicken. *International Food Research Journal* 17: 247-255.
- Wong, S. P., Leong, L. P. and Koh, J. H. W. 2006. Antioxidant activities of aqueous extracts selected plants. *Food Chemistry* 99: 775-783.