

CHARACTERIZATION OF ANTIOXIDANT, ANTIMICROBIAL, ANTICANCER PROPERTY AND CHEMICAL COMPOSITION OF *Phyllanthus urinaria* LINN. LEAF EXTRACT

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Summary

This study was carried out to characterize antimicrobial, antioxidant and anticancer activities of *Phyllanthus urinaria* leaf extract as well as its chemical composition. The main objective of the present study is to reveal the medicinal values of *P. urinaria* leaf. Antimicrobial property of *P. urinaria* leaf extract was revealed by using two fold microdilution method whereas antioxidant activity of the extract was determined with α , α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging method. The anticancer property of the plant extract was revealed through Colorimetric MTT (tetrazolium) assay against human breast adenocarcinoma (MCF-7). Chemical compounds of the plant extract were screening and identified by using gas chromatography-mass spectrometry (GC-MS). The result of the present study showed that the MIC values of the present study ranged from 31.25 to 125 mg/l in which the plant extract was found able to inhibit the growth of all the tested bacterial isolates namely *A. hydrophila*, *E. tarda*, *E. coli*, *Flavobacterium* sp., *Klebsiella* sp., *P. aeruginosa*, *Salmonella* sp., *V. alginolyticus*, *V. cholerae* and *V. parahaemolyticus*. The value of IC_{50} of the plant extract against DPPH was 0.282 ± 0.23 ppt and no anticancer activity was observed in the present study. A total of 9 chemical compounds was successfully identified with Phenol 46.05 % and 2-pentanone, 4-hydroxy-4-methyl-14.69 % were the major compounds. The findings of the present study showed that the antimicrobial and antioxidant properties of the plant extract are promising.

Keywords: antioxidant, anticancer, antimicrobial, chemical compound, *Phyllanthus urinaria* leaf

Introduction

Nowadays, a total of 750 species from the genus of *Phyllanthus* was documented in the literature. As a member of family Euphorbiaceae, this plant was well known for its medicinal properties. Traditionally, the stems, leaves and roots of this plant were used in the treatment of intestinal infection, diabetes, hepatitis, kidney disorder (1) and anticancer (2). Several scientific works were also conducted to reveal the biological properties of this plant. For instance, Cechinel Filho et al. (1996) (3) claimed that this plant possesses antinociceptive activity in mice whereas Shead et al. (1992) (4) revealed that this plant exhibited antiviral against hepatitis B virus. Furthermore, the finding of Unander (1996) (5) revealed that the chemical compound known as angiotensin-converting enzyme was helpful in the treatment of diabetic. Therefore, in the present study, antimicrobial, antioxidant and anticancer activities of *P. urinaria* leaf were characterized to reveal the potential of this plant to be used as medicinal drug.

Materials and Methods

Plant material

The plant sample was purchased from herbal nursery located at Pasir Puteh, Kelantan, Malaysia. The fresh plant sample was oven dried at 37 °C for 4 days. Next, the plant sample was freeze dried prior to extraction using 70% methanol and concentrated at 1 g/ml. Finally, the plant extraction was kept in -20 °C until further use (6; 7).

Bacteria isolates

All bacterial isolates were provided by Universiti Malaysia Kelantan namely *Aeromonas hydrophila*, *Escherichia coli*, *Edwardsiella tarda*, *Flavobacterium* spp., *Klebsiella pneumonia*, *Salmonella typhi*, *Vibrio alginolyticus*, *V. parahaemolyticus*, *V. cholerae* and *Pseudomonas aeruginosa*. These bacteria were isolated from various aquatic animals and kept in tryptic soy agar (TSA) for further uses.

Minimum inhibitory concentration (MIC) determination

The values of minimum inhibitory concentration (MIC) of *P. urinaria* leaf extract against bacterial isolates were determined through a two-fold broth micro dilution method (8; 9; 10). The bacterial isolates were cultured in tryptic soy broth for 24 h at room temperature and the concentration of these cultures were adjusted to 10^9 CFU mL⁻¹ by using physiological saline. The concentration was cross check with a Biophotometer (Eppendorf, Germany). The bacterial suspensions were then inoculated into a microtiter plate that contained a serial dilution of *P. urinaria* leaf extract and positive control. The microplate was then incubated at room temperature for 24 h. The MIC values were defined as the lowest concentration of the *P. urinaria* leaf extract and positive control in the wells of the microtiter plate that showed no visible turbidity after 24 h incubation.

Determination of antioxidant activity with α , α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging method

DPPH radical scavenging method was conducted as described by previous studies with some modifications (11; 12; 13). The assay was carried in a 96 wells elisa plate with three replicates. 5 μ l of the sample (0.5 mg/ml) solution was added into the well followed by 200 μ l DPPH. The absorbance of the sample was recorded by using ELISA reader for ever interval 6 s. The percentage inhibition of DPPH radical was calculated based on the absorbance.

Cancer cell lines

The human breast adenocarcinoma (MCF-7) cell line was derived from Institute of Marine Biotechnology, Universiti Malaysia Terengganu. All the cells were grown in standard cell medium (RPMI 1640) supplemented with 5 % fetal bovine serum in a 5 % CO₂ atmosphere. The cells was then transferred into microplate at the concentration of 1×10^2 cells

per well for cytotoxicity test of the plant extract. At 48 h, proliferation was measured by the MTT colorimetric assay. The IC_{50} value was calculated from the following formula as described Lee et al. (2011) (13):

$$\log_{10}(IC_{50}) = \frac{\log_{10} C_L (I_H - 50) + \log_{10} C_H (50 - I_L)}{I_H - I_L}$$

$$IC_{50} = 10^{\log_{10}(IC_{50})}$$

Where:

I_H : % above 50%

I_L : % below 50%

C_H : High drug concentration

C_L : Low drug concentration

Colorimetric MTT (tetrazolium) assay

Colorimetric MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma, USA) assay was carried out as described by Mosmann (1983). 10 μ l of MTT solution (5 mg/ml) was added to all wells of 96 wells micro plate followed by 4 h incubation at 37 °C. Acid isopropanol was added to all wells for dissolving the dark blue crystals. The microplate plate was then read on an ELISA reader at wavelength 570 nm within 1 h after adding isopropanol (11; 12; 13).

Bioactive compound characterization

The chromatographic procedure was carried out using a Shimadzu QP2010-GC-MS with autosampler. The sample was diluted 25 times with acetone and 1 μ l of sample was injected into a column. A fused silica capillary column HP5-MS (30 m x 0.32 mm, film thickness 0.25 μ m) was used. Helium was the carrier gas, and a split ratio of 1/100 was used. The oven temperature used was maintained at 60 °C for 8 min. The temperature was then gradually raised at a rate of 3 °C per min to 180 °C and maintained at 180 °C for 5 min. The temperature at the injection port was 250 °C. The components of the test solution were identified by comparing the spectra with those of known compounds stored in internal library (11; 12; 13).

Results

The MIC values of the present study ranged from 31.25 to 125 mg/l in which at the concentration of 62.5 mg/l of the plant extract was found can inhibit the growth of *Aeromonas hydrophila*, *Klebsiella* sp., *Salmonella* sp. and *Vibrio alginolyticus* whereas at the concentration of 31.25 mg/l of the plant extract, *Edwardsiella tarda* *Escherichia coli* *Flavobacterium* sp. *Pseudomonas aeruginosa* and *Vibrio cholera* were failed to grow (Table 1). At the concentration of 125 mg/l of the plant extract was able to control the growth of *V. parahaemolyticus* (Table 2). The value of IC_{50} of the plant extract against DPPH was 0.282 ± 0.23 ppt and no anticancer activity was observed in the present study. A total of 9 chemical compounds was successfully identified with Phenol 46.05 % and 2-pentanone, 4-hydroxy-4-methyl- 14.69 % were the major compounds. This was followed by Cyanogen chloride 11.51 %, Butanoic acid 8.05 %, Phosphoric acid, trimethyl ester 5.71 %, 2-Furancarboxaldehyde, 5-(hydroxymethyl)- 4.96 %, Hexadecanoic acid, methyl ester 2.56 %, 1-propanamine, N, 2-dimethyl-N-nitro- 2.41 %, 4,2,8-Ethanylylidene-2H-1-benzopyran, octahydro-2-methyl- 1.36 % and another 3 unidentified compounds 2.70 %.

Bacterial isolates	MIC (mg/l)
<i>Aeromonas hydrophila</i>	62.5
<i>Edwardsiella tarda</i>	31.25
<i>Escherichia coli</i>	31.25
<i>Flavobacterium</i> sp.	31.25
<i>Klebsiella</i> sp.	62.5
<i>Pseudomonas aeruginosa</i>	31.25
<i>Salmonella</i> sp.	62.5
<i>Vibrio alginolyticus</i>	62.5
<i>Vibrio cholera</i>	31.25
<i>Vibrio parahaemolyticus</i>	125

Table 1. Minimum inhibition concentration (MIC) of *Phyllanthus urinaria* leaf extract against bacterial isolates

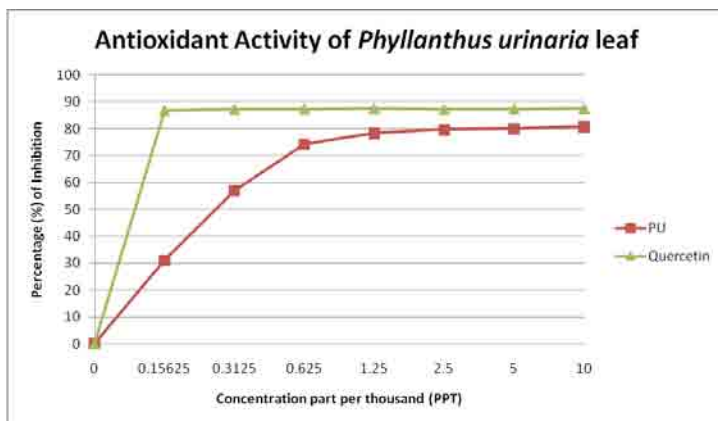


Figure 1. Antioxidant activity of Phyllanthus urinaria leaf

Compound	Compound Composition(%)
Phenol	46.05
2-pentanone, 4-hydroxy-4-methyl-	14.69
Cyanogen chloride	11.51
Butanoic acid	8.05
Phosphoric acid, trimethyl ester	5.71
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	4.96
Hexadecanoic acid, methyl ester	2.56
1-propanamine, N, 2-dimethyl-N-nitro-	2.41
4,2,8-Ethanylidene-2H-1-benzopyran, octahydro-2-methyl-	1.36
3 unidentified compounds	2.70
Total	100.00

Table 2. Compound composition of Phyllanthus urinaria leaf extract

Discussion

The antimicrobial activity of *P. urinaria* was quite well documented in the literature. For instance, Mensah et al. (1990) (14) claimed that this plant was able to control the growth of *Escherichia coli*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Mycobacterium smegmatis*. Another study of Lai et al. (2008) (15) claimed that chloroform and methanol extract of *P. urinaria* was able to inhibit the growth of *Helicobacter pylori* that infected human gastrointestinal. Dabur et al. (2007) (16) was also found that *P. urinaria* exhibited inhibitory activity against various species of bacteria and fungi. Furthermore, several chemical compounds such as Butanoic acid, Phosphoric acid and Hexadecanoic acid were found in the plant extract of the present study that may responsible to the antimicrobial activity of *P. urinaria*. Hence, the antimicrobial property of *P. urinaria* is undoubtedly.

Antioxidant property of *P. urinaria* was well described in the literature. For instance, Xu et al. (2007) (17) revealed that *P. urinaria* possesses huge antioxidant property as this plant contain high phenolic compound. Similar finding was also observed in the present study in which the plant extract exhibit high antioxidant activity against DPPH and almost 50 % of the total compound was identified as phenolic compound. Another study of Fang et al. (2008) (18) also revealed that *P. urinaria* exhibited inhibitory activity against DPPH. The high antioxidant activity of *P. urinaria* was also proved by the study of Chudapongse et al. (2010) (19) in which they found that this plant was effectively can be a hepatoprotective agent in rat. Furthermore, Phenol, Butanoic acid, Phosphoric acid and Hexadecanoic acid were the compounds that successfully isolated and identified in the plant extract where may contribute to the high antioxidant activity of *P. urinaria*.

There are several reports claimed that anticancer property of *P. urinaria* in the literature. For instance, Huang et al. (2003) (20) reported that the extract of this plant was able to control the growth Lewis lung carcinoma cells. This finding was confirmed by the study of Huang et al. (2006) (21) in which they found that this plant exhibited inhibitory activity toward Lewis lung carcinoma cells in the in vivo experiment by using mice as subject. Similar finding was also observed in the study of Chudapongse et al. (2010) (19) where this plant extract can against human hepatocellular carcinoma HepG2 cells in the rat. However, on the other hand, the plant extract in the present study was failed to show any anticancer activity against MCF-7 cells. Furthermore, there is no study reported the anticancer activity of *P. urinaria* towards MCF-7 cells in the literature. Thus, we can conclude that *P. urinaria* may only show the positive response to the selected cancer cells.

In conclusion, from the literature survey and the present study findings, we suggest that *P. urinaria* can be used as a health supplement food as this plant possesses huge antimicrobial and antioxidant activity.

References and further reading may be available for this article. To view references and further reading you must purchase this article.

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