

Characterization of Anticancer, Antimicrobial, Antioxidant Properties and Chemical Compositions of *Peperomia pellucida* Leaf Extract

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Abstract- *Peperomia pellucida* leaf extract was characterized for its anticancer, antimicrobial, antioxidant activities, and chemical compositions. Anticancer activity of *P. pellucida* leaf extract was determined through Colorimetric MTT (tetrazolium) assay against human breast adenocarcinoma (MCF-7) cell line and the antimicrobial property of the plant extract was revealed by using two-fold broth micro-dilution method against 10 bacterial isolates. Antioxidant activity of the plant extract was then characterized using α , α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging method and the chemical compositions were screened and identified using gas chromatography-mass spectrometry (GC-MS). The results of present study indicated that *P. pellucida* leaf extract possessed anticancer activities with half maximal inhibitory concentration (IC_{50}) of 10.4 ± 0.06 μ g/ml. The minimum inhibitory concentration (MIC) values were ranged from 31.25 to 125 mg/l in which the plant extract was found to inhibit the growth of *Edwardsiella tarda*, *Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa* and *Vibrio cholerae* at 31.25 mg/l; *Klebsiella* sp., *Aeromonas hydrophila* and *Vibrio alginolyticus* at 62.5 mg/l; and it was able to control the growth of *Salmonella* sp. and *Vibrio parahaemolyticus* at 125 mg/l. At the concentration of 0.625 ppt, the plant extract was found to inhibit 30% of DPPH, free radical. Phytol (37.88%) was the major compound in the plant extract followed by 2-Naphthalenol, decahydro- (26.20%), Hexadecanoic acid, methyl ester (18.31%) and 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (17.61%). Findings from this study indicated that methanol extract of *P. pellucida* leaf possessed vast potential as medicinal drug especially in breast cancer treatment.

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Introduction

Peperomia pellucida L. HBK or its local name in Malaysia known as ‘ketumpangan air’, are commonly found among Asian countries. It was claimed by the local community that decoction of the plant was useful to treat bone aches and pains (1). The leaf was also used in headache, fever, eczema, abdominal pains and convulsions treatments (2). Elsewhere, this plant served multi-function, including mental disorder treatment in Bangladesh; haemorrhages treatment in Bolivia (3), cholesterol reduction in Brazil (4), and renal problem and uric acid reduction in Guyana and Philipines (5). Some biological properties of *P. pellucida* such as

antibacterial (5), anti-inflammatory (6), analgesic (7), antifungal (8) and anticancer (9) have been studied. Nevertheless, present study was performed to characterize the anticancer, antimicrobial and antioxidant activities of *P. pellucida* methanol extract, together with the analysis of its chemical composition to validate its medicinal potential.

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,

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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.

Bacterial 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 collection were isolated from various aquatic animals and stored in tryptic soy agar (TSA) for further uses.

Determination of anticancer activity

The human breast adenocarcinoma (MCF-7) cell line was derived from the Institute of Marine Biotechnology, University 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 were then transferred into microplate at the concentration of 10² cells per well for cytotoxicity test of the plant extract. At 48 h, proliferation was measured by the colorimetric MTT assay. The half maximal inhibitory concentration (IC₅₀) value was calculated from the following formula (10-12):

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

IC₅₀ = 10 log₁₀ (IC₅₀) Where: I_H : I% above 50% I_L : I% 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) and Lee *et al.* (13-14). Ten microliters of MTT solution (5 mg/ml) was added to all wells of 96 wells microplate followed by 4 h incubation at 37°C. Acid isopropanol was added to all wells to dissolve the dark blue crystals. The microplate was then read with an ELISA reader at wavelength 570 nm within 1 h after addition of isopropanol.

Determination of minimal inhibitory concentration (MIC)

The values of minimal inhibitory concentration (MIC) of *P. pellucida* leaf extract against bacterial isolates were determined through a two-fold broth micro

dilution method (15-16). The bacterial isolates were cultured in tryptic soy broth (TSB) for 24 h at room temperature and the concentration of these cultures were adjusted to 10⁹ CFU ml⁻¹ using physiological saline. The concentration was cross-check with a biophotometer (Eppendorf, Germany). The bacterial suspensions were then inoculated into a microtiter plate which contained serial dilutions of *P. pellucida* leaf extract and positive control. The microtiter plate was then incubated at room temperature for 24 h. The MIC values were defined as the lowest concentration of the *P. pellucida* leaf extract and positive control in the wells of the microtiter plate that showed no visible turbidity after incubation.

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

DPPH radical scavenging method was conducted as described by Blois, Yen and Duh, Brand-William *et al.* and Gadow *et al.* with some modifications (17-20). The assay was carried out in a 96 wells ELISA plate with three replicates. Five microliters of the sample (0.5 mg/ml) solution was added into the wells followed by 200 μl DPPH. The absorbance of the sample was recorded using ELISA reader at every intervals of 6 s. The percentage of inhibition of DPPH radical was calculated based on the absorbance.

Determination of plant extracts chemical composition

Chemical composition of the plant extract was carried out as described by Lee *et al.* (21). The chromatographic procedure was performed using a gas chromatograph mass spectrometer QP2010-GC-MS (Shimadzu, Japan) with autosampler. The sample was diluted 25 times with acetone and 1 μl of sample was injected into a column. A fused silica capillary column HP5-MS (30 m x 0.32 mm, film thickness 0.25 μm) 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.

Results

From present study, anticancer activity of *P. pellucida* leaf extract was anticipated following the

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result of cytotoxicity test against human breast adenocarcinoma (MCF-7) cell line (Figure 1). Viability of cells decreased sharply to 72% when concentration of the extraction was applied at 0.5 µg/ml. Patterns of decrease was rather constant and reach up to 54% viability at the concentration 30 µg/ml. The half maximal inhibitory concentration (IC_{50}) 10.4 ± 0.06 µg/ml was of interest. On top of that, antimicrobial activity of *P. pellucida* leaf extract against ten bacterial isolates was determined whereby the MIC values ranged between 31.25 mg/l and 125 mg/l. Bacterial growth of *Edwardsiella tarda*, *Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa* and *Vibrio cholerae* was inhibited at 31.25 mg/l, while *Klebsiella* sp., *Aeromonas hyrophila* and *Vibrio alginolyticus* was inhibited at 62.5 mg/l. At 125 mg/l of plant extract concentration, bacterial growth of *Salmonella* sp. and *Vibrio parahaemolyticus* was under control (Table 1). *Peperomia pellucida* leaf extract scavenged DPPH with a 30% inhibition at a concentration of 0.625 ppt. Dose-response relationship was observed where the percentage of inhibition slightly increased as the concentration of plant extract increased (Figure 2). Phytol (37.88%) was the major compound in the plant extract followed by 2-Naphthalenol, decahydro-(26.20%), Hexadecanoic acid, methyl ester (18.31%) and 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (17.61%) (Table 2).

Table 1. Minimal inhibitory concentration (MIC) of *Peperomia pellucida* leaf extract against bacterial isolates

Bacterial isolates	MIC (mg/l)
<i>Aeromonas hyrophila</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.	125
<i>Vibrio alginolyticus</i>	62.5
<i>Vibrio cholerae</i>	31.25
<i>Vibrio parahaemolyticus</i>	125

Table 2. Compound composition of *Peperomia pellucida* leaf methanol extract

Compound	Composition (%)
Phytol	37.88
2-Naphthalenol, decahydro-	26.20
Hexadecanoic acid, methyl ester	18.31
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.61
Total	100

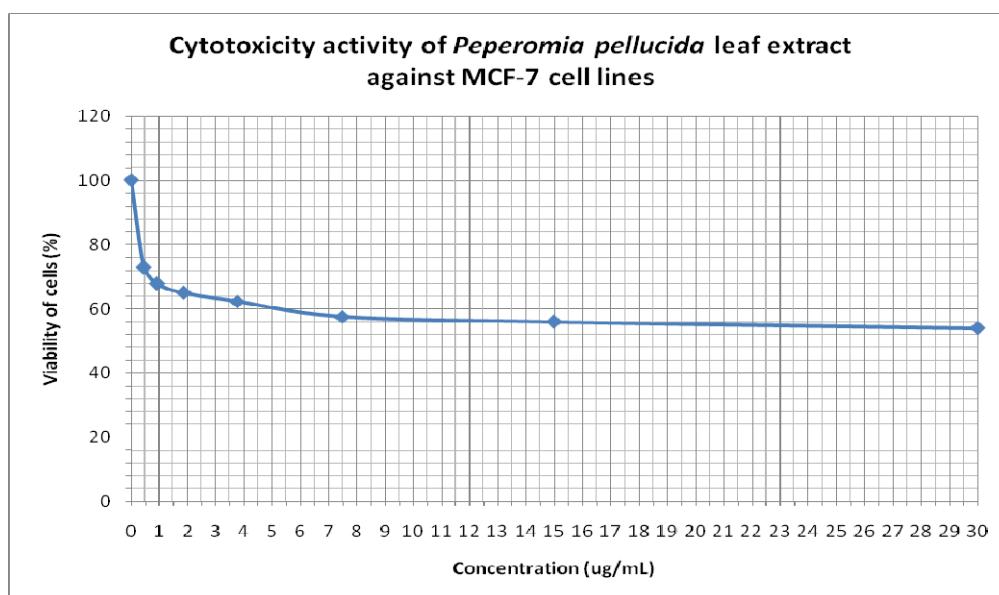


Figure 1. Cytotoxicity activity of *Peperomia pellucida*

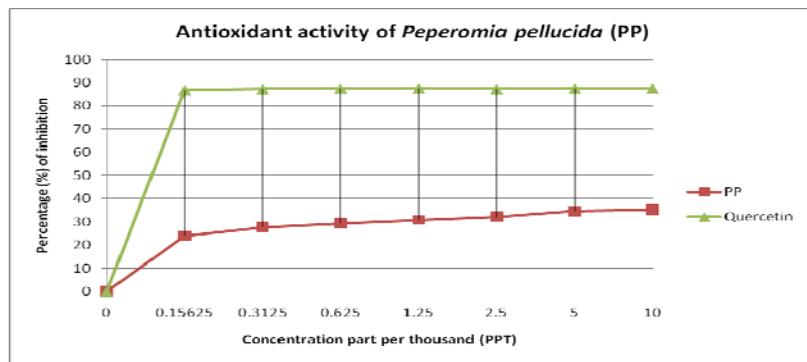


Figure 2. Antioxidant activity of *Peperomia pellucida*

Discussion

The phytochemical compounds of *P. pellucida* greatly contributed to anticancer, antimicrobial and antioxidant activity in present study. Methanol extract of *P. pellucida* leaf was cytotoxic. IC₅₀ 10.4 µg/ml against MCF-7 cell line obtained in present study implied the potential use of *P. pellucida* plant extract in the breast cancer treatment. Similar finding reported by Xu *et al.* claimed that peperomin E isolated from *P. pellucida* leaf extract was found to inhibit the growth of cancer cells (HL-60, MCF-7 and HeLa) with the IC₅₀ ranged from 1.8 to 11.1 µg/ml (9). On the other hand, methanol extract of *P. pellucida* leaf in present study exhibited only moderate antioxidant activity. This was contrary to the studies by Muttee *et al.*, where methanol extract of *P. pellucida* leaf was reported to exhibit high antioxidant activity with IC₅₀ 0.083 ppt (22). Discrepancy of results may due to difference in the extraction method applied. Finely ground plant sample would probably yield more potent plant extract. Chemotypes of flavonoid, alkaloid, diterpenoid and triterpenoid sourced from plants were reported to demonstrate potent cytotoxic activities (23). In the present study, only four compounds were found in the plant extract in which none of the identified compound was reported by Xu *et al.* and Bayma *et al.* (4,9). Phytol, being the major compound in *P. pellucida*, is one of the most important diterpenes and possessed both antimicrobial and anticancer activities (24). Others like hexadecanoic acid, methyl ester and 9, 12-octadecadienoic acid (Z, Z)-, methyl ester were of antioxidant and anticancer property, respectively (24). However, the role of 2-Naphthalenol, decahydro- in the plant extract has yet to be defined and there was lack of studies for comparison. There was difference in the methods employed for the characterization of *P. pellucida* leaf extract chemical composition between present study and Xu *et al.* and Bayma *et al.* (9,4). Although NMR spectroscopic was used in the later

studies, different chemical composition profile was obtained, too.

It is clear that the methanol extract of *P. pellucida* leaf possessed different level of anticancer, antimicrobial and antioxidant properties, which of importance for the development of new therapeutic agents. Further works need to be done in the future to correlate the specific compound with its biological property.

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