Antioxidant Activity Characterization, Phytochemical Screening, and Proximate Analysis of Cermela Hutan (Phyllanthus gomphocarpus Hook. F) Roots and Leaves

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Background: Roots and leaves of the Cermela Hutan (Phyllanthus gomphocarpus Hook. F) plant were studied to determine antioxidant activity, phytochemical compounds, proportion of carbohydrate, crude protein, moisture, ash, fat, total phenolic content (TPC), and total flavonoid content (TFC).

Material/Methods: Ten percent (10%) aqueous extract from both Phyllanthus gomphocarpus roots (PGR) and leaves (PGL) were used in this study. Antioxidant activity characterization by TPC, TFC, Ferric Reducing Antioxidant Power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, and phytochemical screening, as well as proximate analysis from both extracts were analyzed in this study.

Results: Phyllanthus gomphocarpus roots (PGR) and leaves (PGL) tested positive for flavonoid, saponin, tannins, and terpenoids, but PGR showed negative result for anthraquinones. In average weight of 100.0 g dry sample, the carbohydrates, protein, moisture, ash, fat, and energy content in PGR and PGL were 80.9%, 5.5%, 7.8%, 3.4%, 2.4%, and 367 Kcal/100 g, and 66.5%, 14.8%, 10.7%, 6.5%, 1.5%, and 399 Kcal/100g, respectively. Antioxidant assessments using FRAP and DPPH assay showed that PGL extracts possessed higher antioxidant capacity by reducing the ferric ion-TPTZ complex by 0.14 mg/ml ±0.0018 and higher scavenging activity, 83.83% ±0.54 as compared to PGR, 0.07 mg/ml ±0.0035 for FRAP and 62.87% ±1.33 for DPPH, respectively. The total phenolics content was significantly higher in PGL (208.77 mg GAE/g ±3.79) as compared to PGR (27.53 mg GAE/g ±0.42). However, there was no significant different in the total flavonoid contents for PGR (34.8 mg QE/g ±3.12) and PGL (32.43 mg QE/g ±3.92).

Conclusions: Further investigations are suggested to isolate and characterize the other active constituents from this plant in combating diseases.

MeSH Keywords: Antioxidants • Phyllanthus gomphocarpus • Phytochemicals • Proximate analysis

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Background

Plants species of the genus *Phyllanthus* have been traditionally accepted as part of medicinal applications to combat degenerative diseases and have been reported to be beneficial to treating diseases naturally, which has been substantiated by many scientific studies showing that many species of this genus contain various nutraceutical properties that may have positive impacts on human health [1]. Deeper investigations were performed on *Phyllanthus* species to explore nutraceutical action against cancer, pathogenic microorganisms, diabetes, and malaria, as well as having many other beneficial properties [2]. The effectiveness of these plants in treatment of a broad spectrum of diseases may come from the phytochemical compounds they contain. The major active ingredients of most *Phyllanthus* species are tannins, ellagittannins, and flavonoids [3]. Other phytochemical compounds were isolated from *Phyllanthus* species, such as alkaloid, benzenoid, furanolic, tetrpene, and triterpene [1].

Cermela Hutian (*Phyllanthus gomphocarpus* Hook. F.), is a species in the genus *Phyllanthus* of the *Phyllanthaceae* family [4]. It is widely distributed at hilly areas in shady primary forest up to 800 meters altitude and usually found in Peninsular Thailand and Malaysia, Sumatra, and Java [4]. It is a woody plant growing to 3 meters tall and its leaves are ovate-lanceolate shape with small male flowers and large female flowers located between leaves. This plant has light green fruit with a subglobose trilobed capsule shape and size about 1–2 cm diameter, containing small seeds inside [4].

Current knowledge about *P. gomphocarpus* is limited to the botanical aspect and ethnombotanical uses without scientific reports on its phytochemicals or medicinal properties [5]. A decoction from the boiled roots of this plant was traditionally claimed to enhance human health among local traditional practitioners and the Orang Asli community in Malaysia. Thus, this study was conducted to investigate its various phytochemical properties [2]. The effectiveness of these plants in treatment of a broad spectrum of diseases may come from the phytochemical compounds they contain.

Material and Methods

Plant material

*Phyllanthus gomphocarpus* roots and leaves were collected from Felda Keratong 5, Bandar Tun Abdul Razak, Rompin, Pahang Darul Makmur. Authentication of *P. gomphocarpus* (KLU 47925) was carried out in the herbarium of the Rimba Ilmu Botanical Garden, Institute of Biological Sciences, University of Malaya and voucher material for this study was deposited at the same herbarium.

Extraction preparation

The leaves and roots of *P. gomphocarpus* were cleaned immediately to remove any extraneous material, sliced into small pieces and dried in a hot-air oven at 40°C to 50°C. The dried materials were ground into a powder and soaked in distilled water with ratio 1:10 before being boiled at 100°C for 30 minutes [6]. The solvent-containing extract was then decanted and filtered. The filtrates were cooled before being freeze-dried to obtain a greenish powder from roots and dark-brown powder from leaves. All the crude extracts were weighed and dissolved in dimethyl sulfoxide (DMSO), to form stock solutions prior the assay and were then kept in a refrigerator.

Phytochemical screening

Phytochemicals screening was performed by using standard procedures [7,8].

Flavonoids

About 0.5 gram of the extract was heated with 10 ml of ethyl acetate in a steam water bath for 3 minutes. The mixture was then filtered using Whatman No. 1 filter paper and the filtrates were shaken with 1 ml of ammonia solution. A change in color to yellow indicates the presence of flavonoids in the samples.

Saponins

In a test tube, 0.5 gram of the extract was added with 5 ml of distilled water. The solution was shaken and a stable persistent froth was observed. Then 3 drops of olive oil was mixed with the froth and shaken vigorously. The formation of the emulsion indicates the presence of saponins in the samples.

Tannins

Approximately 0.5 gram of the extract was boiled in 10 ml of water. The mixture was then filtered and a few drops of 0.1% ferric chloride were added into the filtrate. A brownish-green or blue-black formation indicates the presence of tannins.

Terpenoids

In a test tube, 2 ml of chloroform was added with 0.5 gram of the extract. Then 3 ml of concentrated sulfuric acid was added to form a layer. Reddish-brown coloration of the interface indicates terpenoids.

Flavonoids

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Tannins

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**Anthraquinones**

About 0.5 gram of extract was boiled with 10 ml of sulfuric acid and quickly filtered while hot. The filtrate then was shaken with 5 ml of chloroform. The chloroform layer was pipetted out into another test tube and added to 1 ml of dilute ammonia. Color changes of the solution indicate anthraquinones.

**Radical scavenging activity**

Radical scavenging activity (RSA) was determined using a stable DPPH radical and expressed as the percentage of the inhibition activity after 30 minutes of incubation. Plant extract (0.5 ml) was added to 0.45 mM solution of DPPH in ethanol. Absorbance at 517 nm was determined after 30 minutes, and the percentage of inhibition activity was calculated from \[ (A_0 - A_1) / A_0 \times 100 \], where A0 is the absorbance of the control and A1 is the absorbance of the extract/standard [2,9].

**Ferric reducing antioxidant power (FRAP) assay**

The FRAP assays were carried out as previously described by Baghat et al. (2014) [10]. Reductions of ferric tripyridyl triazine (Felli TPTZ) complex to ferrous form were monitored by measuring the changes in absorbance at 593 nm. FRAP reagent was prepared by mixing the TPTZ (2.5 ml) with 20 mM ferric chloride (2.5 ml) and 0.3 mM acetate buffer (25 ml). Then plant extracts (100 µl) were added into the reagent, incubated at room temperature for 4 minutes, and the absorbance was read at 593 nm.

**Total phenolic content (TPC)**

The Folin-Ciocalteu (FC) method was used to determine total soluble phenols content: 500 µL of Folin-Ciocalteu reagent: water (1:1) reacted in assay tubes with 100-µl aliquots of the aqueous extract for 6 minutes. After neutralization was applied with 1.25 mL of 20% Na₂CO₃, the volume was adjusted to 10 ml with distilled water. Mixtures were shaken on a vortex and left in darkness for 2 hours to achieve stabilization with absorbance read at 765 nm. A standard curve was prepared with gallic acid to express total soluble phenols content as mg of gallic acid equivalents (GAE)/g of extract powder [11].

**Total flavonoid content (TFC)**

The aluminum chloride method was used for the determination of the total flavonoid content of the root sample extracts. The aliquots of extract solutions made by adding methanol to reach a volume of 3 ml. Then 0.1 ml AlCl₃ (10%), 0.1 ml Na-K tartrate and 2.8 ml distilled water were added sequentially. The test solutions were shaken vigorously and the absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin [11]. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of extract powder.

**Proximate analysis**

The proportion of protein, moisture, carbohydrate, energy, ash, and fat in dried samples of *P. gomphocarpus* roots and leaves were estimated in accordance with the standard in-house methods described by the Association of Official Analytical Chemists (AOAC) 16th edition. The analyses were carried out at UNIPEQ Sdn. Bhd., Universiti Kebangsaan Malaysia and are reported as percentages per 100 grams of samples.

**Statistical analysis**

All data were analyzed using Statistical Package for Social Science (SPSS) program version 20. The significance of the differences between means was established by subjecting the results to analysis of variance (ANOVA) followed by post hoc Tukey test for FRAP and DPPH analysis. Significance difference between means for TPC and TFC was established by 2-group comparison using the t test. All data are presented as average ± standard deviation (SD). Values of p<0.05 were regarded as statistically significant.

**Results**

**Phytochemical screening of *P. gomphocarpus***

The phytochemical screening of *P. gomphocarpus* showed the presence of flavonoids, tannins, saponins, and terpenoids in roots and leaves. Anthraquinones were detected in leaves (PGL) but not in roots (PGR) (Table 1).

**Proximate analysis of *P. gomphocarpus***

The proximate analysis of *P. gomphocarpus* roots and leaves is presented in Table 2. The analysis revealed that carbohydrate

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**Table 1. Phytochemical constituents of *P. gomphocarpus* roots and leaves.**

| Test          | Roots | Leaves |
|---------------|-------|--------|
| Flavonoids    | +     | +      |
| Saponins      | +     | +      |
| Tannins       | +     | +      |
| Terpenoids    | +     | +      |
| Anthraquinones| –     | +      |
was the largest contributor to proximate weight for roots (80.9%) and leaves (66.5%). Protein, ash, and moisture were found to be higher in leaves (14.8%, 6.5%, and 10.7%) than in roots (5.5%, 3.4%, and 7.8%), respectively. The measured fat content differed from that of earlier results. Fat content was found to be higher in roots (2.4%) compared to leaves (1.5%). In contrast, the energy content of leaves (399 Kcal/100 g) was higher than in the roots (367 Kcal/100 g).

### Table 2. Proximate analysis of *P. gomphocarpus* roots and leaves.

| Constituents     | Roots   | Leaves  |
|------------------|---------|---------|
| Protein (g/100 g)| 5.5     | 14.8    |
| Fat (g/100 g)    | 2.4     | 1.5     |
| Carbohydrate (g/100 g)| 80.9 | 66.5     |
| Ash (g/100 g)    | 3.4     | 6.5     |
| Moisture (g/100 g)| 7.8  | 10.7    |
| Energy (Kcal/100 g)| 367.0 | 399.0   |

### 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

Figure 1 represents the scavenging activity of BHT, Vitamin C, *P. gomphocarpus* root and leaf extracts. Samples with (*) are significantly different (p<0.05). Values are expressed as mean ± SD.

### Ferric reducing antioxidant power (FRAP) activity

Figure 2 represents the ferric reducing antioxidant power (FRAP) ability of BHT, and the samples of *P. gomphocarpus* roots and leaves. The standard antioxidant BHT demonstrated the highest antioxidant power, with FRAP value of 0.35 mg/ml ±0.024, and was significantly higher compared to roots and leaves of *P. gomphocarpus*. The antioxidant power in leaves was significantly higher compared to the root sample, with FRAP value of 0.14 mg/ml ±0.0018 and 0.07 mg/ml ±0.0035, respectively.

### Total phenolic contents (TPC)

Total phenolic content (TPC) of *P. gomphocarpus* roots and leaves was determined by using a standard plot of gallic acid (y=3.7186x – 0.0012, R²=0.9992) (Figure 3). The analysis revealed that phenolic content was significantly higher (p<0.05) in leaves compared to roots, with 208.77±3.79 and 27.53±0.42 mg of gallic acid equivalent (GAE) per gram of the extract, respectively.
Ported that terpenoids can suppress tumor cell proliferation in roots and leaves of *P. gomphocarpus*. Therefore, it appears that this compound high potential as an antioxidant [23].

Anthraquinones in leaves of *P. gomphocarpus* suggest the potential of this plant as a source of anti-viral drugs. Sydikis et al. (1991) reported that anthraquinones from plants may inhibit the adsorption and replication of herpes simplex virus (HSV). Results from phytochemical screening show that *P. gomphocarpus* roots and leaves contain various beneficial compounds. Thus, these findings suggest that this plant has potential as a source of important bioactive compounds and may be useful as a source of treatment of various diseases.

**Total flavonoid content (TFC)**

Total flavonoid content (TFC) of *P. gomphocarpus* roots and leaves were determined by using standard plot of quercetin ($y=0.041X + 0.0603, R^2=0.9965$) (Figure 4). The analysis revealed that flavonoid content was not significantly higher ($p>0.05$) in roots compared to leaves, with 34.8±3.12 mg and 32.43±3.92 mg of quercetin equivalent (QE) per gram of the extract respectively.

**Discussion**

Phytochemical screening of *P. gomphocarpus* roots and leaves revealed the presence of flavonoids, saponins, tannins, and terpenoids. However, anthraquinones were not detected in the roots sample. These phytochemical compounds are known to be biologically active and useful in treating various diseases [12]. Flavonoids are bioactive polyphenolic compounds found in most plants and cannot be synthesized or produced by the humans [13]. It was found to be effective in controlling various biological activities and is anti-inflammatory, anti-antigiotic, antimicrobial, antioxidant, reduced hypertension, and has anti-cholesterol properties [14–17]. *P. gomphocarpus* roots and leaves were reported to contain saponins, which have anti-diabetic, antioxidant, anti-pyretic, and anti-microbial therapeutic properties [18].

Igbinosa et al. (2009) found that saponins may be useful in managing inflammation. Similar to the flavonoids, tannins are phenolic compounds found in plants, acting as primary antioxidants or free radical scavengers [7]. However it was seen to be more effective in treating inflamed and ulcerated tissue [19]. Siquera et al. (2012) revealed the anti-diarrheal and anti-microbial properties of tannins. Terpenoids were also found in roots and leaves of *P. gomphocarpus*. Goto et al. (2010) reported that terpenoids can suppress tumor cell proliferation and apoptosis-inducing activity. Therefore, it appears that this plant may have potential as a source of medicine for prevention and treatment of cancer. It was also proven to reduce cholesterol and effectively treat cardiovascular diseases [20].

DPPH testing provides important information on the ability of *P. gomphocarpus* roots and leaves to scavenge stable free radicals. The strength of antioxidants compounds present in this plant against free radicals was determined by their hydrogen donating ability to donate proton ions to DPPH [21]. When electrons from an antioxidant and DPPH become paired-off, DPPH solution is then decolorized, as the color changes from deep violet to light yellow [7]. The degree of discoloration indicates the percentage of radical scavenging ability of antioxidants in this plant. The scavenging ability of roots and leaves of *P. gomphocarpus* were less potent compared to standard antioxidants such as BHT and vitamin C. Presence of flavonoids and tannins that act as primary antioxidants or free radical scavengers in both roots and leaves are likely responsible for their scavenging ability against DPPH [7]. However, the scavenging ability of leaves was 20.96% more potent than roots. This finding reveals that the hydrogen donating ability in leaves was significantly higher compared to roots, suggesting that leaves are superior to roots in terms of radical scavenging abilities.

Ferric reducing antioxidant power (FRAP) is a measure of the reductive ability against free radicals as evaluated by the transformation of ion Fe (III) to Fe (II) in the presence of the sample extracts [22]. There were significant difference between the level of reducing ability in PGL and PGR, suggesting that PGL have more reducing ability compared to a PGR sample. Phenolic compounds donate their hydrogen ion, which then reduces the Fe (III) to Fe (II) and thereby neutralizing the free radicals [21,22].

Total phenolic content (TPC) determination by using Folin-Ciocalteu method was carried out to determine the level of total phenolic in PGL and PGR. Results showed that PGL possess significantly more TPC than in PGR, consistent with the antioxidant activity that was found to be higher in PGL. Higher percentages of scavenging activity and the reducing ability of PGL are associated with the higher amount of TPC, perhaps due to the presence of conjugated ring structures and hydroxyl group that give this compound high potential as an antioxidant [23].
Different results was obtained in total flavonoid content (TFC), with higher amounts of TFC in PGR compared to PGL. However, the differences between these 2 types of extract were not significant. Flavonoid was found to possess broad-spectrum chemical and biological activities, including free radical scavenging activity, but results may vary due to the assay method [2,24]. Thus, presence of flavonoids suggests their roles in the antioxidant activity of PGR and PGL.

The proximate analysis of PGR and PGL was carried out in this study by using ratio of constituents per 100 gram of dried sample. Result in Table 2 revealed that PGL possess more protein, ash, moisture, and energy compared to PGR. However, more fat and carbohydrate were found in PGR compared to PGL.

Considering the increasing interest of the food industry in herbs with high phenolic contents [2,23], this finding may indicate that PGL and PGR also can be used as a source of nutrition in maintaining health status and preventing various diseases.

Conclusions

Our results strongly suggest that *P. gomphocarpus* roots and leaves are potential sources of natural antioxidants and nutrients for the treatment of various diseases induced by free radicals. Further investigations are needed to isolate and characterize the other active substances in this plant and to explore their potential in combatting diseases.

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