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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *PILEA MELASTOMOIDES* (LONDOU OPIAU), A NATIVE PLANT OF SABAH

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ABSTRACT. *Pilea melastomoides (P. melastomoides) belongs to the family Urticaceae and is commonly known as Melastome Clearweed. It is locally referred to as "Londou opiau" by the indigenous people of Sabah, Malaysia. Traditionally, this plant has been used to treat pregnancy-related conditions, gastritis, infertility, skin disorders, and injuries, although no scientific evidence currently supports these claims. The objective of this study is to identify the phytochemical constituents of P. melastomoides and evaluate its antioxidant activity. Phytochemical constituents were identified using qualitative analysis (tannins, phlobatannins, saponins, flavonoids, terpenoids, and cardiac glycosides) as well as quantitative analysis of total phenolic content (TPC) and total flavonoid content (TFC). Antioxidant activity was assessed using an in vitro DPPH assay. P. melastomoides's stems ethanolic extract content tannins, flavonoids and terpenoids. The TPC and TFC values be found of 11.21 ± 0.2 mg GAE/g and 1.48 ± 0.07 mg CAE/g, respectively. The IC_{50} value be found of 2.4 mg/mL against DPPH radicals. This study serves as first report on P. melastomoides on phytochemical constituents and antioxidant activity from a native plant from Sabah, East Malaysia.*

INTRODUCTION

Pilea melastomoides is one of rarest native plants that can be found in Kg. Narawang, Ranau of Sabah, East Malaysia (500 to 700 meters above sea level). This plant traditionally used as herbal drink to treat pregnant, gastric, infertility, skin problem, and injury. Additionally, this plant known as 'Pohpohan' by local Indonesian and its primary agricultural crop for the Tamansari Village community (Permatasari *et al.*, 2024; Adhiningsih *et al.*, 2022). Traditionally use as side dish (Sriwahjuningsih, 2022), wood production (Idami *et al.*, 2022; Khairunnisa et al., 2024), and medicinal plants. Best of our knowledge, one article mentions that *P. melastomoides* have medicinal values with antipyretic properties due to the presence of phytochemical compounds (Peralta, 1983). Therefore, due to limited data on pharmacological properties, the investigation is demanded to identify the phytochemical constituents and antioxidant through scientific validation.

The important pharmacological investigation in plant is the phytochemical compounds possessed with antioxidant activity. Antioxidant activity is the ability of the compound to capture free radicals causing oxidative stress through *via* neutralization of reactive oxygen species (ROS) (Gnanaraj

et al., 2027). Common antioxidant agents are vitamin C and E. Phytochemical compounds with antioxidant properties derived from secondary metabolites such as tannins, phylobatannins, saponins, flavonoids, terpenoids, and cardiac glycoside. Phytochemical compounds with antioxidant properties have ability to donate electron to free radical ions and stabilize it from further radical chain reactions (Ringgit *et al.*, 2025). Additionally, these compounds important as defense system against disease or predator (Hbika *et al.*, 2025). Therefore, the determination of potential phytochemical compounds from local plan, *P. melastomoides* create a wide opportunity to develop a natural drug with minimal side effects.

To date, insufficient data on pharmacological evidence of *Pilea melastomoides*, but the best clues we could gather are based on the traditional knowledge and application. Previous researches focusing on species diversity (Permatasari *et al.*, 2024; Susiarti *et al.*, 2021), source management (Permatasari *et al.*, 2024; Adhiningsih *et al.*, 2022; Sriwahjuningsih, 2022; Khairunnisa *et al.*, 2024), traditional application (Iskandar et al., 20230, metagenomic analysis (Yonantiko et al., 2024), and photocatalytic application (Yonantiko *et al.*, 2024). Therefore, the purpose of this study is to serve as a first report on *P. melastomoides* related to phytochemical constituents and antioxidant activity. The research finding offering a significant data of pharmacological value of *P. melastomoides* from native plant of Sabah for further investigation.

MATERIALS AND METHODS

Chemicals and Reagents

Ferric chloride ($FeCl_3$), ammonia (NH_3), Folin-Ciocalteu's reagent, sodium nitrite (NaNO2), aluminum chloride ($AlCl_3$), sulfuric acid (H_2SO_4), acetic acid ($FeCl_3$), DPPH (2,2-Diphenyl-1-picrylhydrazyl-hydrate), and sodium carbonate (Na_2CO_3) purchased from Sigma-Aldrich (Burlington, MA, USA); and hydrochloric acid (HCl) and chloroform ($CHCl_3$) from Merck (Darmstadt, Germany).

Plant Collection and Extraction

Pilea melastomoides's stems were collected in July, 2009 at Kg. Narawang, Ranau of Sabah, East Malaysia. The sample was washed, cut into small size, dried, and grinded using heavy duty blender into coarse powder form. Next, 20 g sample was macerated into 600 mL ethanol at a 1:30 (w/v) sample-to-solvent ratio stirring for 72 hours using magnetic stirrer, filtered, and evaporated using a rotary evaporator with a water bath temperature of 40 °C at a rotation speed of 60 rpm. The extracts kept at -20°C freezer for further analysis.

Phytochemical Constituents (Qualitative Assay)

Tannins were identified by a color change to brown or dark blue after adding a few drops of 1.0% ferric chloride ($FeCl_3$). Phlobatannins were detected by forming a precipitate when 1% hydrochloric acid (HCl) was added to the ethanolic extract. Saponins were identified by mixing the extract with distilled water, shaking vigorously to produce froth, and adding olive oil to check for emulsion formation. Flavonoids were confirmed by a yellow coloration after adding 1% ammonia (NH_3) solution. Terpenoids were detected by mixing the extract with chloroform, then adding sulfuric acid, which turned the solution reddish-brown. Cardiac glycosides were identified by forming a brown ring after mixing the extract with acetic acid, $FeCl_3$, and sulfuric acid.

Phytochemical Constituents (Quantitative Assay)

Total phenolic content (TPC) was measured by mixing a 200 µL aliquot of 1 mg/mL extract with 1.5 mL of Folin-Ciocalteu's reagent and incubating it in the dark for 5 minutes. Then, 1.5 mL of 60 g/L sodium carbonate was added, and the mixture was incubated for 90 minutes. TPC was determined spectrophotometrically at 725 nm using gallic acid as the standard and expressed as mg GAE/g of stems. Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay. A 0.5 mL extract was mixed with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite, then incubated

for 6 minutes. After adding 0.15 mL of 10% aluminum chloride and incubating for another 6 minutes, 2 mL of 4% sodium hydroxide was added. Total volume of 5 mL distilled water added and left for 15 minutes and measured at 510 nm using catechin as a standard (mg CAE/g).

Antioxidant Activity

Antioxidant activity of *Pilea melastomoides*'s stems measured using DPPH (2,2-Diphenyl-1-picrylhydrazyl-hydrate). A 2.7 mL of 0.06 mM DPPH solution added to respective sample concentration ranges of 10-2400 µg/mL and kept for 60 minutes in the dark condition. The absorbance was measured using spectrophotometer at 517 nm. The scavenging activity in the presence of DPPH radicals was calculated as Equation 1 below:

$$\text{DPPH scavenging activity, \%} = [(A_{control} - A_{sample})/A_{control}] \times 100\% \quad (1)$$

$A_{control}$ is an absorbance of gallic acid and A_{sample} is an absorbance of stems. The scavenging activity of extract sample obtained based on the half inhibition concentration (IC_{50}).

Statistical Analysis

All data were obtained from expression of standard deviation (±S.D) from triplicate of number of sample (n = 3). Graph was plotted to determine the IC_{50} value from standard calibration curve (y= mx + C) and regression (r^2 = 0.999 ± 0.1) from Microsoft Excel software.

RESULTS AND DISCUSSION

Phytochemical Constituents (Qualitative assay)

Table 1. shows the phytochemical constituents in *Pilea melastomoides*'s stems extracted using ethanol solution. Six compounds been evaluated including tannins, phylobatannins, saponins, flavonoids, terpenoids, and cardiac glycosides. This assay based on the qualitative assay which it refers to the presence and absence of the compounds in the plant. The presence compound extracted using ethanol solution labelled as positive (+) while no compound presence marked as negative (-). Based on the qualitative assay, three out of six (3/6) assays detected compounds including tannins, flavonoids and terpenoids. Tannins derived from alkaloids identified based on the chemical reaction of ferric chloride ($FeCl_3$), changed the extract solution to brownish or dark blue. Additionally, ammonia (NH_3) turned the solution into yellow colour, indicated the presence of flavonoids. The chemical reaction between chloroform and sulfuric acid turned the extract solution into reddish brown due to the presence of terpenoids in the sample. Although phlobatannins, saponins, and cardiac glycosides are major compounds that reported commonly be found in plant but in this study, there was no chemical reaction of the assay towards phlobatannins, saponins, and cardiac glycosides suggested to be absence in stem part.

Table 1. Phytochemical constituents of *Pilea melastomoides*'s stems.

Phytochemical constituent	Ethanolic extraction
Tannins	+
Phlobatannins	-
Saponins	-
Flavonoids	+
Terpenoids	+
Cardiac glycosides	-

Presence, +; absence, -.

Phytochemical Constituents (Qualitative Assay)

Total phenolic content (TPC)

The amount of phenol and flavonoids compounds in *Pilea melastomoides*'s stems from Sabah measured based on quantitative assay on total phenolic content (TPC) and total flavonoid content (TPC). The TPC value in plant extract measured by Folin-Ciocalteu's reagent and sodium carbonate. This reaction determined based on the common indicator for phenolic compounds in plant, gallic acid. Similarly, catechin used as a baseline concentration of flavonoid compound in plant extract based on the chemical reaction of sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), and sodium hydroxide (NaOH). Table 2. shows the amount of phenolic and flavonoid compounds in 1 mg/mL *P. melastomoides*'s stems based on ethanolic extraction. Based on the table, the stem contains high phenolic compounds than flavonoid compounds. The concentration of TPC in *P. melastomoides*'s stems measured according to the indicator, gallic acid. At absorbance of 725 nm, the concentration phenolic compounds of 11.21 ± 0.2 mg GAE/g. The total phenolic content (TPC) in *Pilea melastomoides*'s stems is lower than leaves which agreement with previous finding by Anderwulan *et al.* (2012).

Table 2. Total phenolic content (TPC), total flavonoid content (TFC) of *Pilea melastomoides*'s stem.

Parameter	Content
TPC	11.21 ± 0.2 mg GAE/g
TFC	1.48 ± 0.07 mg CAE/g

Milligrams of gallic acid weight, mg GAE/g; milligrams of catechin weight, mg CAE/g.

Total flavonoid content (TFC)

The amount of TFC be found of 1.48 ± 0.07 mg CAE/g at absorbance of 510 nm based on catechin standard. The presence of phytochemical constituents in *P. melastomoides* varies across different plant parts, except for flavonoids. The leaves contain a higher concentration of phytochemical compounds compared to the stems. In this study, *Pilea melastomoides*'s stems contain tannins, flavonoids and terpenoids while according to Anderwulan *et al.* (2012), they identified the presence of flavonoids (luteolin, quercetin, and kaempferol), phenolic compounds (chlorogenic acid, caffeic acid, and ferulic acid), and carotenoid (β-Carotene) in *P. melastomoides*'s leaves. The presence of phytochemical compounds is important in pharmaceutical industries as well as epidermal cell secretion (Bidlack, 2000). Similarly, the flavonoid content in the present study such as a stem lower than previous report, a leaf. In stem the flavonoid content be found of 1.48 ± 0.07 mg CAE/g compared to leaves (2.34 mg/100 g) (Andarwulan *et al.*, 2012). The authors identified the flavonoid based on sum of specific flavonoid compounds detected (luteolin, quercetin, and kaempferol). Although this finding showed lower TFC level form stem part, but the diversity of flavonoid content among the plants need to evaluate due to flavonoid reported possessed with antioxidant activity (Andarwulan, 2010). In this experiment, the TPC ethanolic extraction be found of 11.21 ± 0.2 mg GAE/g while 121.51 ± 1.64 mg GAE/100 g obtained from leaves, while methanol:acetic acid solvent by Anderwulan *et al.* (2012) of 18.57 ± 0.40 mg/100g. This result suggested the phytochemical constituents in plant part influences the composition of phytochemical compounds including extraction yield (Islam *et al.*, 2008), and polarity solvent (Maier *et al.*, 2010; Ringgit *et al.*, 2024a;2024b).

Antioxidant Activity

Phenolic and flavonoid compounds have been reported to exhibit antioxidant activity. Antioxidant activity refers to the ability of plant-derived compounds to scavenge free radical ions and disrupt radical chain reactions that contribute to oxidative stress in living cells. However, the presence of phenolic and

flavonoid compounds in plant extracts, as determined by qualitative and quantitative assays, does not necessarily guarantee their scavenging activity. Therefore, a common antioxidant assay, the DPPH assay, was performed to validate this claim. Notably, the ethanolic extract of *P. melastomoides* stems exhibited a scavenging activity of 19.47% against DPPH radicals. Determining the actual concentration of bioactive compounds is crucial in antioxidant assays, as precise concentration levels influence the efficacy of plant extracts in neutralizing free radicals. In this study, the ethanolic extract of *P. melastomoides* stems required a concentration of 2.4 mg/mL to achieve 50% inhibition of free-radical activity, as indicated by the half-maximal inhibitory concentration (IC_{50}) value (Table 3). The concentration calibration curve ranged from 10 to 2400 mg/mL and was represented by the equation $y = 5.6367x + 1.065$, with an R^2 value of 0.9705. There was limited information on scavenging activity of *Pilea melastomoides*. The best of our knowledge, we compare the scavenging activity plants species under same genus (Pilea). It is worth to note that, in this research (*P. melastomoides's* stem) reported low IC_{50} value of 2.4 mg/mL compared to *Pilea microphylla* (all plant part: 23.15 µg/mL) and *Pilea symmeria* (leaves: 194.86 ± 3.87 µg/mL) reported by Prabhakar *et al.* (2007) and Lalremtluangi *et al.* (2025), respectively. This result suggested that antioxidant agents' presence in *P. melastomoides*'s stem have scavenging capacity even in low concentration.

Table 3. IC_{50} of *Pilea melastomoides*'s stem.

Parameter	Activity
DPPH (IC_{50})	2.4 mg/mL

Half inhibition concentration, IC_{50}.

CONCLUSION

In conclusion, *Pilea melastomoides* content tannins, flavonoids and terpenoids with TPC and TFC values of 11.21 ± 0.2 mg GAE/g and 1.48 ± 0.07 mg CAE/g, respectively. These compounds possess with antioxidant activity (2.4 mg/mL) against DPPH radicals. It is worth to note that, this is the first report on potential phytochemical compounds with antioxidant activity derived from *P. melastomoides*, medicinal plant of Sabah, Eastern Malaysia. This information provides an important finding to develop medicinal plants by utilizing the potential native plant source.

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