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Phytochemical Screening and Antioxidative Potentials of *Plectranthus Amboinicus* Leaves Extract

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Abstract

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defence system, plays a critical role in the development of non-communicable diseases (NCDs) such as cardiovascular disease, diabetes, and cancer. These diseases are increasingly prevalent in Malaysia, contributing significantly to national morbidity and mortality. While synthetic antioxidants are widely used, their potential health risks have prompted an increased interest in safer, plant-derived antioxidants as alternatives. Plectranthus amboinicus, also known as Cuban oregano, or pokok bangun-bangun, is traditionally used to treat respiratory conditions, digestive disorders, and fever. Although the plant's biological activities are widely studied, there is still limited research done on the P. amboinicus plant from Malaysia. This study aimed to investigate the phytochemical constituents and antioxidant activity of P. amboinicus leaves collected in Terengganu, Malaysia. In this study, the crude methanol and aqueous P. amboinicus extract were prepared using a combination of maceration and ultrasound-assisted extraction techniques. Then, phytochemical screening, total phenolic, and total flavonoid content were conducted to identify bioactive substances. The antioxidant activity of the leaves was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay. Findings revealed the presence of flavonoids, phenolics, coumarins, and cardiac glycosides in the extract. Quantitative analysis demonstrated that the methanol extract exhibited higher flavonoid (21.27 ± 4.85 mg QE/g) and phenolic content (59.96 ± 18.48 mg GAE/g) when compared to the aqueous extract. The DPPH scavenging activity is also significantly higher in the methanol extract, with the IC50 of 76.23 ± 32.74 µg/mL when compared to the aqueous extract (112.04 ± 45.02 µg/mL). Similar trends were observed in FRAP assay, with the value of 0.38 ± 0.025 mM Fe2+/g extract for methanol and 0.31 ± 0.13 mM Fe2+/mg extract for aqueous, respectively. The study highlighted the presence of a significant amount of phenolics and flavonoids in the extract, emphasising their significant impact on the antioxidant properties of P. amboinicus extract. In conclusion, P. amboinicus exhibits antioxidant activity and has the potential to be used as a natural antioxidant.

Keywords

Plectranthus amboinicus, Phytochemicals, Total Flavonoid, Total Phenolic, Antioxidant.

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Introduction

Oxidative stress occurs when the production of reactive oxygen species (ROS) overwhelms the body's antioxidant defence system. Generated during normal metabolism or triggered by external factors such as ultraviolet (UV) radiation, pollutants, and tobacco smoke, excess ROS can damage lipids, proteins, and nucleic acids[1]. This imbalance is strongly associated with the pathogenesis of chronic diseases, including cardiovascular disorders, diabetes, cancer, and neurodegenerative conditions[2].

To counteract oxidative stress, organisms rely on a complex defence system consisting of enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), and non-enzymatic compounds, including ascorbic acid, glutathione (GSH), flavonoids, and carotenoids[3]. While synthetic antioxidants have been widely used, concerns about their toxicity and safety have fueled interest in natural alternatives that are safer, more effective, and economically sustainable[4].

Higher plants are particularly rich sources of antioxidant phytochemicals, and several species within the Lamiaceae family have been extensively studied. Among them, *Plectranthus amboinicus*, also known as Indian borage, stands out as a traditional medicinal plant with notable therapeutic potential[5]. This aromatic, succulent herb is widely used in ethnomedicine for treating ailments such as respiratory disorders, fever, epilepsy, and infections.

Phytochemical investigations have revealed that *P. amboinicus* contains diverse bioactive metabolites, including flavonoids such as apigenin, luteolin, and salvigenin. Experimental studies have highlighted its pharmacological activities, encompassing antioxidant, anti-inflammatory, antimicrobial, antimutagenic, antiviral, antifungal, antitumorigenic, antiepileptic, radioprotective, and neuropharmacological effects[5,6]. Importantly, acute toxicity studies in animal models suggest that *P. amboinicus* is safe, with no side effects, and exhibits relatively low acute oral toxicity, further supporting its therapeutic potential[6,7,8].

Despite these promising findings, variations in the phytochemical composition and antioxidant activity of *P. amboinicus* have been reported, which may be influenced by geographical origin, environmental conditions, and extraction methods. Therefore, the present study aims to evaluate the phytochemical profile and antioxidant activity of *P. amboinicus* leaves collected in Terengganu, Malaysia. The findings are expected to provide new insights into the therapeutic potential of this medicinal herb and contribute to the growing body of evidence supporting the use of natural antioxidants in health promotion.

Materials and Methods

Collection of Plant Materials

P. amboinicus leaves were cultivated and collected in Kuala Nerus, Terengganu. The plant was authenticated and preserved in a herbarium for future reference at the Faculty of Bioresources & Food Industry (FBIM), University Sultan Zainal Abidin (UniSZA), with a voucher specimen UniSZA/A/000000691. Prior to plant extraction, the leaves were thoroughly washed with tap water three times to remove any dust or dirt particles. Prior to processing, the leaves were placed in a Memmert oven to dry at 50°C for 3-5 days or until the leaves were completely dry and the weight remained constant9. Following that, the material was ground into a fine powder by using a mechanical grinding machine and stored in a dark container for the extraction process.

Plant Extraction

The leaves were extracted using a combination of maceration and ultrasound-assisted extraction (UAE) to produce high-yield content and preserve their antioxidant compounds. For this purpose, the sample was subjected to a maceration process in three cycles, using methanol and aqueous solutions, respectively, for 24 hours10. The mixture was stirred constantly by using a hotplate stirrer. After 24 hours, the mixture was then sonicated using a sonicator for 30 minutes to enhance the extraction of bioactive compounds. Then, the plant mixture was filtered using a tea filter and further filtered with Whatman filter paper No. 1. A rotary evaporator (Eyela OSB-2100 model) was used to concentrate the filtrate product at 60°C to obtain a methanolic crude extract. Meanwhile, a freeze-dryer (Christ Alpha 1-2 LSC basic Model) was then used to lyophilize the crude extract for 72 hours.

Qualitative Phytochemical Analysis

Qualitative phytochemical tests were conducted to detect the presence of alkaloids, steroids, tannins, saponins, cardiac glycosides, flavonoids, quinones, coumarin, phenolics, and terpenoids based on previously described methods by Nor et al. (2019)[11].

Total Flavonoids Content (TFC)

The total flavonoid content in *P. amboinicus* leaves was determined using aluminium chloride by Wan et al., (2021) with minor modifications[12]. A range of quercetin concentrations (0.0625-1000 mg/mL) was prepared with 100% methanol and served as a standard. 25 µL of different concentrations of quercetin was mixed with 375 µL of 75% ethanol, 25 µL of 10% AlCl3. 25 µL sodium acetate and 700 µL of distilled water. 25 µL of sample extracts (methanol and aqueous) were also mixed with the same solution as the standard. The mixture of different concentrations of standard and sample was incubated at room temperature for 30 minutes. Then, the absorbance was read at 415 nm using a microplate reader. The total flavonoid content (TFC) was expressed as the quercetin equivalent (QE) in milligrams per gram of dry weight (mg QE/g DW).

Total Phenolic Content (TPC)

The total phenolic content in *P. amboinicus* leaves was determined by the colourimetric method by Wan et al., (2021) with minor modifications[12]. A series of gallic acid concentrations (0.0625-1000 mg/mL) was prepared in 100% methanol and used as a standard. 25 µL of different concentrations of gallic acid was mixed with 725 µL of distilled water and 125 µL of Folin & Ciocalteu reagent. 500 µL of 25% sodium carbonate. 25 µL of sample extracts (methanol and aqueous) was also mixed with the same solution as the standard. The mixture of different concentrations of standard and sample was incubated at room temperature for 1 hour and 30 minutes. Then, the absorbance was read at 765 nm using a microplate reader. The total flavonoid content (TFC) was expressed as the gallic acid equivalent (GAE) in milligrams per gram of dry weight (mg GAE/g DW).

2,2′-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH assay was carried out using the method of Wan et al., (2021) with minor modifications[12]. DPPH assay was performed in 96-well plates. Butylated hydroxytoluene (BHT) and gallic acid (GA) were used as positive controls for the DPPH assay. The DPPH solution was prepared by dissolving 3.8mg of DPPH in 95ml of 100% methanol. The prepared DPPH was then subsequently added to various concentrations of the extracts. To do this, 150 µL of extract of *P. amboinicus* was mixed with 150 µL of 0.04 mg/ml DPPH. Then, the mixture was incubated at room temperature for 30 minutes. DPPH free radical reduction was then measured by reading the absorbance at 517nm using a microplate reader. Experiments were repeated in three independent assays. Radical scavenging activity (% of inhibition) was calculated based on the following calculation.

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Inhibition (%) = [Blank reagent – (Sample-Blank sample)] / (Blank reagent)

The sample is the absorbance of the current sample after dilution with DPPH solution. The blank reagent consists of a combination of sample solvent and DPPH solution. The blank sample is a combination of sample dilution with solvent. The results for each concentration were then plotted to measure the IC50 [13].

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed by using the method by Nor et al. (2020) with minor modifications [11]. Firstly, 1mM standard stock solution was prepared using Iron (II) sulfate with distilled water. Then, the ferrous sulfate solution was prepared with various concentrations (0-1.0 mM) with distilled water. After that, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM hydrochloric acid, and 20 mM iron (III) chloride were mixed in a 10:1:1 ratio to make FRAP reagent. Then, 1.8 mL of FRAP reagent was mixed with 150 µL of distilled water and 50 µL of the sample that was prepared in triplicate. The mixture was then incubated for 4 minutes in a water bath at 37 °C. The absorbance of each sample was measured at 593 nm. The FRAP activity was expressed as millimoles of Fe (II) per gram of sample, based on the calibration curve prepared with FeSO4 .7H2O.

Statistical Analysis

All data were analysed using GraphPad Prism version 9 and Excel Software. Every test extract was performed in triplicate (n = 3), and the values are represented as the mean and standard deviation (SD). One-way ANOVA was used for statistical analysis of the results. The differences between the IC50 of the samples were compared using Tukey's test in GraphPad Prism. Lastly, the differences between the methanol and aqueous extracts of the sample for TPC, TFC, and FRAP assays were calculated using an independent T-test.

Results

Qualitative phytochemical screening

The results of the qualitative phytochemical screening of *P. amboinicus* leaf extracts are summarised in Table 1. The methanolic extract contained flavonoids, phenolics, quinones, coumarins, and cardiac glycosides, whereas the aqueous extract revealed the presence of flavonoids, coumarins, cardiac glycosides, and steroids. Notably, phenolics and quinones were exclusively detected in the methanolic extract, while steroids were only identified in the aqueous extract.

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Table 1: The phytochemical constituents present in the plant extracts.

Extracts	*P. amboinicus* (Methanol extract)	*P. amboinicus* (Aqueous extract)
Tannin	-	-
Saponin	-	-
Flavonoid	+	+
Phenolic	+	-
Terpenoid	-	-
Alkaloid	-	-
Quinone	+	-
Coumarin	+	+
Cardiac Glycoside	+	+
Steroid	-	+

Quantitative determination of phytochemical constituents

The phenolic content (TPC) and the total flavonoid content (TFC) of the extract were determined using the Folin-Ciocalteu and aluminum chloride method, respectively. For TPC, the value was quantified using a calibration curve (y = 0.001x + 0.049, R2 = 0.982) of gallic acid (0–1000 µg/mL) and expressed in mg gallic acid equivalents (GAE) per gram crude extract weight. As shown in Table 2, the TPC is 59.96 ± 18.48mg GAE/g and 40.18 ± 15.76 mg GAE/g for the methanol and aqueous extracts, respectively, with no statistical significance observed for either extraction method. For the TFC, the value was calculated based on a calibration curve (y = 0.002x + 0.045, R2 = 0.999) of quercetin (Q) (0–1000 µg/ml) and expressed in mg quercetin equivalents (QE) per gram crude extract weight. As shown in Table 2, the TFC in methanol extracts (21.27 ± 4.85 mg QE/g) is significantly higher when compared to aqueous extract (7.78 ± 1.73 mg QE/g) ($p < 0.05$).

Table 2: Quantification of total phenolic content (TPC) and total flavonoid content (TFC) in methanol and aqueous *P. amboinicus* leaves extract.

Extracts of *P. amboinicus* leaves	Total phenolic content (mg GAE/g of extract)	Total Flavonoids content (mg QE/g of extract)
Methanol Extract	59.96 ± 18.48	21.27 ± 4.85*
Aqueous Extract	40.18 ± 15.76	7.78 ± 1.73

Values are the mean of triplicate determination ± standard deviation; GAE-Gallic acid equivalents. QE-Quercetin equivalents.
*significant differences ($p < 0.05$) when compared to aqueous extract for total flavonoid content (TFC).

Antioxidant activity of P. amboinicus leaves extract

The percentage of DPPH radical scavenging activities of *P. amboinicus* extracts and positive control, gallic acid (GA), and butylated hydroxytoluene (BHT) against various concentrations (0–1000 µg/mL) are depicted in Figure 1. As shown in the figure, the percentage of DPPH inhibition for BHT, aqueous, and methanolic extracts of P. amboinicus leaves, increased gradually as the concentration increased. The percentage of DPPH inhibition for GA increases until it stops and reaches a plateau.

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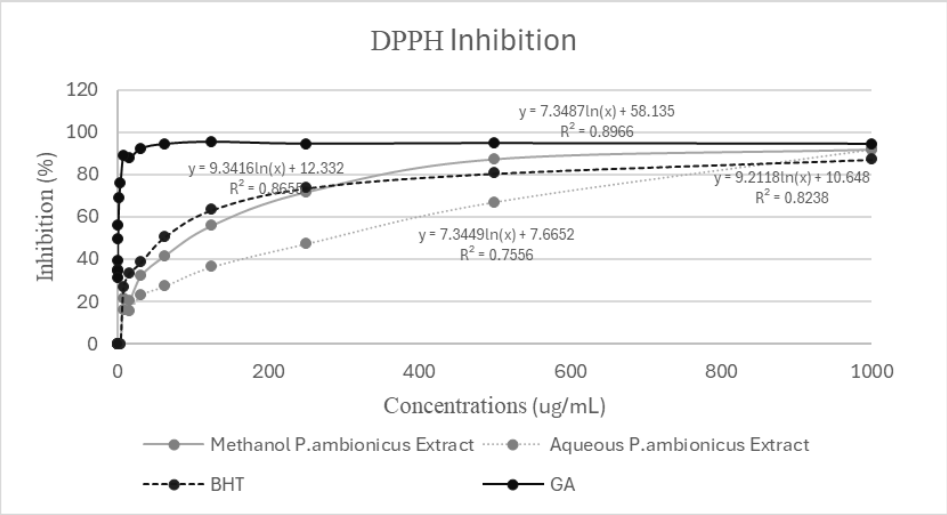

Figure 1: The percentage of DPPH inhibition of *P. amboinicus* (aqueous extract, methanol extract) and positive controls (gallic acid and BHT).

Table 3 demonstrates the IC50 value of the methanol and the aqueous extract of *P. ambionicus* leaves, together with the positive control butylated hydroxytoluene (BHT) and gallic acid (GA). Findings show that GA had the lowest IC50 value, 0.37 ± 0.29 µg/mL, which indicated its potent free radical scavenger ability, followed by BHT, methanolic extract, and aqueous extract of *P. amboinicus*, with IC50 values of 53.78 ± 7.01 µg/mL, 112.04 ± 45.02 µg/mL and 112.04 ± 45.02 µg/mL, respectively. Findings indicated that the IC50 value of *P. ambionicus* methanol and aqueous extract were significantly higher when compared to GA ($p < 0.05$), but no significant difference in the IC50 value of both extracts when compared to BHT.

Table 3: IC_{50} score for methanol and aqueous *P. amboinicus* leaves extracts, BHT, and gallic acid.

Sample	IC_{50} (µg/mL)
Methanol Extract	76.23 ± 32.74
Aqueous Extract	112.04 ± 45.02
BHT	53.78 ± 7.01
Gallic acid (GA)	0.37 ± 0.29*

Values are the mean of triplicate determination ± standard deviation. * $p < 0.05$ when compared to methanol and aqueous extract of *P. ambionicus*.

Table 4 shows the antioxidant capacity of methanol and aqueous *P. ambionicus* extract using the FRAP assay. The capacity of the extracts to reduce ferric (III) iron to ferrous iron is used to assess antioxidant activity in this study. As indicated in Table 4, the methanol extract of *P. amboinicus* leaf possessed higher antioxidant power with 0.38 ± 0.03 mM/g than the aqueous extract with a value of 0.31 ± 0.13 mM/g. However, there are no significant differences in the antioxidant capacity observed for both extraction methods ($p > 0.05$).

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Table 4: Total antioxidant activity of methanol and aqueous *P. amboinicus* leaves extract.

Extracts of *P. amboinicus* leaves	FRAP Assay (*m*M Fe^{2+}/g extract)
Methanol Extract	0.38 ± 0.03
Aqueous Extract	0.31 ± 0.13

Values are means of triplicate determination ± standard deviation.

Discussion

Medicinal plants are well known for producing diverse bioactive compounds with antioxidant properties that protect the human body against oxidative stress. Antioxidants act by neutralising free radicals, thereby preventing oxidative damage associated with degenerative diseases. Phytochemicals such as flavonoids, phenolic acids, and polyphenols found in medicinal plants play a particularly important role as free radical scavengers, inhibiting oxidative processes linked to chronic illnesses[14].

Plectranthus amboinicus (Mexican mint/Indian borage) is a promising medicinal plant with strong antioxidant potential. Its leaves are rich in secondary metabolites, including essential oils, terpenes, phenolics, flavonoids, tannins, alkaloids, esters, and steroids. Traditionally, it is consumed as a vegetable and used in folk medicine[15]. Although numerous studies have been conducted on the biological activities of *P. ambionicus*, the findings vary based on geographical location, weather, and different phases of plant material collection and extraction methods or solvents. Additionally, there is limited research on the *P. amboinicus* plant from Malaysia. Thus, the present study aims to evaluate the phytochemical profile and antioxidant activity of *P. amboinicus* leaves collected in Terengganu, Malaysia.

Phytochemical screening revealed that both methanol and aqueous extracts of *P. ambionicus* contained flavonoids, coumarins, and cardiac glycosides. Phenolics and quinones were detected only in methanol extracts, while steroids were unique to aqueous extracts. These secondary metabolites are biologically active and contribute significantly to the pharmacological properties of medicinal plants[16,17]. For quantitative analysis, findings confirmed that methanol extracts contained higher levels of phenolics and flavonoids compared to aqueous extracts. The total phenolic content (TPC) of the methanol extract was slightly higher, consistent with previous studies[18,19,20]. The high TPC suggests that phenolic compounds contribute substantially to the antioxidant capacity of *P. amboinicus*. Similarly, total flavonoid content (TFC) was significantly higher in the methanol extract (21.27 ± 4.85 mg QE/g) compared to the aqueous extract (7.78 ± 1.73 mg QE/g). This was also higher than values reported by Laila et al. (2020) and Sulaiman et al. (2018), underscoring the influence of solvent type, geographical origin, and extraction conditions on phytochemical yield[21,22].

Previous studies have consistently shown that methanol, particularly in aqueous mixtures, yields higher total phenolic and flavonoid contents than aqueous or acetone solvents alone[23,24,25]. Findings suggest that methanol, being highly polar, penetrates plant cell walls more efficiently and can dissolve phenolic compounds better than less polar solvents. Moreover, the use of ultrasound-assisted extraction (UAE) methods in this study further enhances the leachable phenolics and flavonoid compounds from the extract. Consistent with previous reports, UAE significantly enhanced the yield of both total phenolics and total flavonoids compared to conventional extraction across a range of optimized conditions[26,27]. A review on the UAE on plant bioactive compounds also supports that the UAE's efficiency is due to enhanced plant cell wall disruption and improved solvent penetration[28].

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Phenolics and flavonoids are known for their antioxidant, anti-inflammatory, anti-tumor, and cardioprotective properties[29]. Their antioxidant action derives from redox properties that enable them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators[30,31]. The high concentrations of these compounds in *P. amboinicus* methanol extract likely underpin its strong antioxidant activity.

The antioxidant potential was assessed using DPPH and FRAP assays. In the DPPH assay, gallic acid (GA) which serves as positive control exhibited the lowest IC50 (0.366 ± 0.290 µg/mL), confirming its strong radical scavenging activity, followed by butylated hydroxytoluene (BHT) (53.78 ± 7.02 µg/mL), methanol extract (106.30 ± 30.54 µg/mL), and aqueous extract (112.04 ± 45.02 µg/mL). Although the methanol extract demonstrated slightly stronger activity than the aqueous extract, the difference was not statistically significant. Both extracts were comparable to BHT, though significantly less potent than GA ($p < 0.05$). These findings corroborate earlier studies by Kumaran and Karunakaran (2006) and Gurning (2020), who reported strong radical scavenging activity in *P. amboinicus* extracts, with higher efficacy in methanol[32].

The FRAP assay further confirmed the antioxidant potential, with the methanol extract of *P.amboinicus* showing a higher ferric reducing activity (0.379 ± 0.025 mM Fe^{2+}/g) than the aqueous extract (0.307 ± 0.132 mM Fe^{2+}/g). Comparable findings were reported by Swamy et al. (2017), where methanol extracts demonstrated greater reducing capacity than acetone extracts. Collectively, these results suggest that methanol is a more effective solvent for extracting phenolic and flavonoid compounds from *P. amboinicus* leaves, which in turn contributes to stronger antioxidant activity[33]. The observed variations between studies highlight the influence of solvent type, extraction conditions, and plant origin on phytochemical content and bioactivity.

Conclusion

This study demonstrated that *Plectranthus amboinicus* leaves collected from Terengganu are a rich source of bioactive secondary metabolites, particularly phenolics and flavonoids, which contribute significantly to their antioxidant potential. Methanol extracts yielded higher levels of phenolic and flavonoid compounds compared to aqueous extracts, which corresponded with stronger antioxidant activity as confirmed by DPPH and FRAP assays. These findings align with earlier reports yet provide new region-specific evidence, addressing the current scarcity of scientific data on *P. amboinicus* from Terengganu, Malaysia. The results contribute to the growing body of knowledge on medicinal plants by confirming the phytochemical richness and antioxidant potential of this locally available species, thereby supporting its traditional use and highlighting its value as a natural source of health-promoting compounds. Importantly, this study highlights the importance of regional phytochemical profiling, as variations in environmental and geographical factors can influence bioactivity.

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Conflict of Interest Disclosure

None to declare.

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