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Antioxidant Potential of *Chloranthus erectus* (Chloranthaceae) from various solvents extract

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ABSTRACT

Chloranthus erectus is a herbaceous plant that has been used as a medicinal plant in several regions such as China and Southeast Asia. Although it possesses valuable medicinal properties, till now there is not much research has been carried out on the medicinal properties of this plant and the knowledge of this plant is limited among the research fertility. Therefore, this study aimed to identify the phytochemicals, total phenolic content (TPC), and antioxidant activity of leaf and twig of *C. erectus* in various solvents extract (hexane, petroleum ether, chloroform, ethyl acetate, and methanol). Phytochemical screening of extracts showed the presence of alkaloids, flavonoids, terpenoids, saponins, quinones, glycosides, and steroids. The highest phenolic content for leaf and twig samples was determined from the methanolic (9.64 ± 0.15 µg GAE/g) and hexanoic extract (7.39 ± 0.27 µg GAE/g), respectively. Meanwhile, the highest antioxidant activity was reported from the methanolic extract of both leaf (88.36 ± 0.24%) and twig (91.25 ± 0.10%) samples. Hence, the results of the study can be concluded that *C. erectus* has the potential to become a good natural antioxidant and the information from this study can be utilized by the communities as well as other researchers.

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1 Introduction

The consumption of herbal medicinal products by the public has increased significantly. Many medicinal practitioners have been focusing on the use of natural antioxidants instead of synthetic antioxidants. Synthetic antioxidants, such as Tempol, Probucol, and Transcrocetinate have various negative effects on human health and causing cancer and inducing premature senescence (Kornienko et al. 2019). Therefore, researchers have turned their attention toward medicinal plants and herbs and produced a safer and more natural antioxidant.

C. erectus belong to the family of Chloranthaceae, locally known as Sambau Paya in Peninsular Malaysia, Langut Langut in Sarawak, and Totol in Sabah has a long history of use as a medicinal plant. This plant has an average height of 3 m with broad shining dark green leaves (20 cm x 8 cm) and produces white bud-like flowers (Kiew et al. 2010). This shrub is found in tropical climate regions such as China, Eastern Himalayas, and Southeast Asia. Its habitat is primarily in a mountain forest at a lower altitude, where the plant can grow under the shade of trees and nearby a river that has moist soils (Kiew et al. 2019).

Although it is highly used as an herbal cure by folk traditional healers and modern herbalists in many indigenous communities of Asian countries, it is generally a lesser-known medicinal herb in the region, which is almost entirely unknown in other countries. A few studies have been done on different species from the Chloranthaceae family. For example, Zhang et al. (2016) have reported that *C. henryi* has been used as an alternative supplement in improving blood circulation. However, insufficient scientific research has been made to discover *C. erectus* chemical constituents and to know its pharmacological actions. Therefore, the present study was conducted to identify phytochemicals, total phenolic content (TPC), and antioxidant activity of leaf and twig of *C. erectus* from various solvents extract (hexane, petroleum ether, chloroform, ethyl acetate, and methanol). The expected output from this study is information about the potential of unexplored valuable local medicinal plant of *C. erectus* collected from Taman Negara Ledang, Johor for the early stage of drug discovery.

2 Materials and methods

2.1 Plant collection

Fresh *C. erectus* plant parts were collected from Taman Negara Ledang, Johor (2.3312589, 102.6125548). The collected plant samples were sent to Forest Research Institute Malaysia (FRIM) in Kepong, Malaysia for authentication (PID 160820-12).

2.2 Preparation of plant extract

The leaf and twigs of the plant were separated and gently washed with tap water and air-dried for a week. Dried samples were ground to a fine powder by using a mixer grinder. The dried powdered sample of the leaf (200 g) and twig (80 g) was extracted sequentially with five different solvents namely hexane, petroleum ether, chloroform, ethyl acetate, and methanol by maceration technique for 72 hours, respectively. The extracts were then gravitationally filtered using Whatman No. 1 filter paper and the filtrates were evaporated to dryness using a rotatory evaporator. The percentage yield of each extract was calculated and recorded by using the below equation and sample extracts were kept refrigerated at -20^{o}C until further use (Balasubramaniam et al. 2020).

% Yield of extract =

$$\frac{\text{Weight of extract residue after solvent removal (g)}}{\text{Weight of dried sample powder (g)}} \text{ X } 100$$

2.3 Phytochemical screening

The presence of phytochemicals from each solvent extract of leaf and twig was carried out using the standard procedure of Keshav et al. (2019) with slide modification to identify the presence of flavonoids, terpenoids, and saponins. While for the identification of quinones, steroids, and glycosides, a method by Shaikh and Patil (2020) was used.

2.4 Determination of total phenolic content (TPC)

The TPC for *C. erectus* leaf and twig extracts was determined by using the Folin-Ciocalteu method given by Madiha et al. (2016) with a slight modification. For this, a total of 0.5 ml of sample was mixed with Folin-Ciocalteu reagent and left in dark for 5 minutes. This was followed by the addition of 1.5 ml of 7.5% of sodium carbonate and then left incubated for 30 minutes in the dark. Finally, the absorbance was read by using UV-Vis spectrophotometer at 725 nm. The same process was repeated for gallic acid with various concentrations (10, 25, 50, 75, and 100 µg/ml) to construct a calibration curve. The results were expressed as µg gallic acid equivalent (GAE)/g dry weight of extract (Romes et al. 2019).

2.5 DPPH scavenging activity

Exactly 0.2 ml with a concentration of 50 µg/ml of the sample was pipetted out into a test tube. To this tube, 3 ml of 0.1 mM DPPH solution was added, mixed well, and incubated in a dark room for 30 minutes. The absorbance was read at 517 nm against a blank solution which is distilled water. Ascorbic acid was used as a standard reference. The percentage inhibition was calculated for the sample and standard (Masuku et al. 2020). Using the below-mentioned equation the percentage inhibition was calculated:

$$\% \text{ Inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \text{ X } 100$$

Where Abs is the absorbance

2.6 Statistical analysis

The data were expressed as a mean value ± standard deviation of triplicates (n=3). The statistical analysis used a one-way ANOVA test, while the significance of differences between means was determined by using the Games-Howell test at $p \leq 0.05$ by using Statistical Package for Social Science (SPSS) software.

3 Results and discussions

3.1 Extraction of *C. erectus* plant extract

In this study, the maceration extraction technique was applied, in which the sample was first extracted with a non-polar solvent and then continue with more polar solvents successively. This is because a single solvent would not be able to extract all phytochemical and antioxidant compounds from the plant material due to the chemical nature of the compounds. Therefore, by using different solvents and increasing the polarity of the solvent from non-polar to polar, a wide range of compounds can be extracted (Nawaz et al. 2019). Results presented in Table 1 revealed the summary of the extraction of *C. erectus* leaf and twigs.

Results presented in Table 1 suggested that among the tested five extracts, methanolic extract yielded the highest phytochemical compound consistently compared to the other four extracts from both leaf and twig samples which were 4.29% and 14.79%, respectively. The differences in yield percentage among the solvents were due to the polarity of the solvent used during extraction. According to Do et al. (2014), the polarity of the solvent affects the yield of crude extracts. This also indicates that the chemical compounds in this plant are mostly polar, thus, producing a high yield percentage in the polar solvent. Table 1 also shows that the extracts produced are mostly semi-solid and viscous.

3.2 Phytochemical screening

Phytochemical screening tests were conducted to detect the presence of phytochemical compounds in five different solvents. Each extract may consist of different types of compounds therefore some of the extracts showed negative results on certain tests. Table 2 summarises the results of phytochemical screening that had been conducted on *C. erectus* leaf and twig extracts demonstrating the presence of various phytochemicals such as alkaloids, flavonoids, terpenoids, saponins, quinones, and steroids.

The phytochemical qualitative analysis displayed that the hexane and ethyl acetate extracts recorded the highest phytochemical compounds in both leaf and twig samples compared to other extracts. These findings can be supported by a study done by Vivi Mardina et al. (2020) in which they used both ethyl acetate and hexane solvent for extraction and recorded that ethyl acetate recovered more chemical compounds compared to hexane. This demonstrated that the polarity of solvent can affect the phytochemical compound yield. In addition, the twig sample exhibits a wide range of phytochemical compounds in phytochemical analysis. This finding was in line with a study reported by Wang et al. (2015) in which they managed to recover various phytochemical compounds from the same part of the plant in other different species of Chloranthus plant.

The presence of flavonoids indicated that this species has the potential to be used as remedies and as a natural antioxidant agent. Zhang et al. (2016) studied the constituents of many plant species belonging to the Chloranthaceae family. In the study, they managed to isolate flavonoids from another *Chloranthrus* plant namely *C. multistachys*. A study done by Xu et al. (2020) also discovered flavonoids from another *Chloranthus* species

Table 1 The extraction summary of leaf and twig extracts from *C.erectus* on various solvents

Plant Part	Solvent	Weight of Dried Leaf Powder (g)	Physical Properties	Weight of Extract Residue After Solvent Removal (g)	Percentage Yield (%)
Leaf	Methanol	200	Semi-solid	8.76	4.29
	Ethyl Acetate		Semi-solid	3.49	1.71
	Chloroform		Semi-solid	3.12	1.53
	Petroleum Ether		Semi-solid	3.21	1.57
	Hexane		Semi-solid	7.17	3.51
Twig	Methanol	80	Jelly-like	11.83	14.79
	Ethyl acetate		Semi-solid	0.69	0.86
	Chloroform		Semi-solid	3.35	4.19
	Petroleum ether		Semi-Solid	0.83	1.04
	Hexane		Semi-solid	1.14	1.43

*Semi-solid physical properties represent a sticky and viscous-like texture; Jelly like physical properties represent a squishy cube-like structure

Table 2 Phytochemical screening of *C.erectus* leaf and twig extract

Test	Methanol		Ethyl acetate		Chloroform		Petroleum ether		Hexane	
	Leaf	Twig	Leaf	Twig	Leaf	Twig	Leaf	Twig	Leaf	Twig
Alkaloid	+	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	+	-	+	-	+	+	+
Terpenoids	-	+	-	+	-	+	-	+	-	+
Saponins	+	+	-	+	-	+	-	-	-	+
Quinones	-	-	+	+	+	-	+	+	+	+
Steroids	-	+	+	+	-	+	+	+	-	+

(+) = Presence, (-) = Absence

named *C. henryi*. These findings, therefore, strongly suggest high antioxidant activity in *Chloranthus* spp, which is commonly associated with the presence of polar phenolic and flavonoid compounds.

Presence of the terpenoids in plant extract also plays an important role in antioxidant activity. A study conducted by Mohandas and Kumaraswamy (2018) stated that the presence of terpenoids in a considerable amount could contribute to high antioxidant activity. However, based on Table 2, there were no terpenoids present in both leaves or twig samples. This could be due to the method used in this study which is qualitative analysis. Compared to quantitative analysis, the qualitative analysis only detects the compound's presence and appeared to lack sensitivity and specificity, which could impact the result obtained (Tzima et al. 2018). Therefore, further analysis is recommended to quantify the density/number of terpenoids in this plant.

3.3 Total phenolic content (TPC)

In this study, the total phenolic content (TPC) for *C. erectus* leaf and twig samples is tabulated in Table 3. The TPC was expressed in terms of gallic acid equivalent (µg GAE/g dry weight) by using the equation based on the calibration curve where y = 0.0109x + 0.0377, R^2 = 0.9801.

Based on the results obtained in Table 3, the highest TPC was recorded for a methanolic extract for the leaf sample by 9.64 ± 0.15µg GAE/g dry weight while for the twig sample, hexane extract managed to record 7.39 ± 0.27µg GAE/g dry weight. Due to its polar nature, the amount of phenolic compound in methanolic leaf samples was influenced by the solvent polarity thus giving a higher value. However, the hexanoic twig sample managed to achieve the highest total phenolic content compared to other extracts. This could be because of the presence of other compounds in the hexane extract that could influence the TPC of the twig extract. A study carried out by Gema et al. (2020) reported that different compounds such as terpenes and saponin can interact with the complex phenol structure which in turn interferes with the phenolic content quantification. These results can also conclude that this plant has the potential as a good natural antioxidant agent. This is because phenolic compound possesses redox properties that could help in neutralizing free radicle molecules (Zheng and Wang 2001; Huyut et al. 2017). As of today, there is no study has been reported on the total phenolic content of *C. erectus* or any other Chloranthus genus plants except for one study done by Xu et al. (2020) in which they managed to obtain TPC content ranging from 4.36 – 19.64 mg GAE/g dry weight from the n-butanol extract of *C. henryi*.

Table 3 Total phenolic content of *C. erectus* leaf and twig extracts

Extract	Total phenolic content (µg GAE/g dry weight)	
	Leaf	Twig
Methanol	9.64 ± 0.15	6.07 ± 0.03
Ethyl acetate	4.92 ± 0.04	6.36 ± 0.15
Chloroform	2.34 ± 0.08	2.83 ± 0.07
Petroleum ether	3.00 ± 0.02	0.94 ± 0.38
Hexane	0.47 ± 0.02	7.39 ± 0.27
Standard 2 (Gallic Acid)	99.60 ± 0.86	

*Values are represented as mean value ± standard deviation

Table 4 Antioxidant activity of *C. erectus* leaf and twig extracts

Extract	Concentration (µg/ml)	DPPH Scavenging Activity (%)	
		Leaf	Twig
Methanol	50	88.36 ± 0.24	91.25 ± 0.10
Ethyl acetate		55.43 ± 0.20	13.32 ± 0.45
Chloroform		31.87 ± 0.77	13.27 ± 0.38
Petroleum ether		11.00 ± 3.37	33.40 ± 1.33
Hexane		12.65 ± 4.01	30.34 ± 0.52
Standard (Ascorbic Acid)		95.10 ± 0.21	

*Values are represented as mean value ± standard deviation

3.4 DPPH scavenging activity

In the case of the antioxidant activity of *C. erectus*, the results presented in table 4 summarized the result of DPPH scavenging activity in percentage.

Results presented in Table 4 revealed that the highest antioxidant activity can be observed from the methanol extract of both leaves (88.36 ± 0.24%) and twig (91.25 ± 0.10%). Few studies suggested that antioxidant activity is associated with the maturity of the plant itself. A study done by Kuntorini et al. (2022) on the maturity effect and antioxidant activity of leaves and fruits of *Rhodomyrtus tomentosa* suggested that young leaves have high antioxidant activity compared to old leaves. Some studies suggest that plant antioxidant activity could be affected by the presence of chlorophyll in the sample. According to Simao et al. (2013), the antioxidant level is high when there is a low presence of chlorophyll in a sample. This could be supported by the results shown in Table 4 and when the methanolic extract of the twig sample managed to record the highest antioxidant activity compared to the methanolic leaf sample due to the reason twigs have less chlorophyll compared to the leaf. It is also noted that the methanol extract of twig showed the highest antioxidant activity and it is as good as ascorbic acid (95.10 ± 0.21%). Xu et al. (2020) reported that the antioxidant activity of another different Chloranthus species suggested the presence of a phenolic compound that could show a good antioxidant property. Other than that, there are not many studies that have reported on antioxidant activity for this plant or any associated species. In short, *C. erectus* has the potential and can be applied as an antioxidant agent.

Conclusion

Recent findings exhibited that both leaf and twigs extracts of *C. erectus* possess various phytochemicals such as alkaloids, flavonoids, saponins, quinones, and steroids. Among the tested various extracts, the methanolic extract showed the highest TPC for the leaf sample while for the twig sample, hexane extract displayed the highest TPC compared to other extracts. In addition, both samples of methanolic extracts displayed the highest antioxidant activity.

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