

Identification of Bioactive Compounds, Quantitative Measurement of Phenolics and Flavonoids Content, and Radical Scavenging Activity of *Lygodium circinnatum*

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ABSTRACT Studies have proven that oxidative stress plays a major contribution towards many diseases. The ability of antioxidants status to recover from certain diseases is shown to be important for the improvement of human's health. *Lygodium circinnatum* (Lygodiaceae) is used by local Sabahan in treating various diseases. In this study, the presence of bioactive compounds in the aqueous extract of *L. circinnatum* were chemically tested and has resulted in the detection of these compounds; alkaloids, flavonoids, tannins, phenolics compound and glycosides. Total content of phenolics and flavonoids were determined by using quantitative measure. In-vitro antioxidant activity was investigated by the method for total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity using UV-VIS spectrophotometer. The content of phenolics was 31.84 ± 0.24 mg/ml gallic acid equivalent and flavonoids were 63.5 ± 1.67 mg/ml catechin equivalent. The IC_{50} value of *L. circinnatum* in DPPH assay was 143.76 μ g/ml whereas IC_{50} of standard ascorbic acid was 39.43 μ g/ml. The results obtained have shown such important phytochemical properties present in *L. circinnatum* and is expected to be beneficial in treating diseases where oxidative stress is implicated.

KEYWORDS: Oxidative stress; *L. circinnatum*; aqueous extraction; phenolics; flavonoids; radical scavenging activity

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INTRODUCTION

Oxidative process is essential for the continuity of the cells to take part in the regulation of body system. When the body system is overwhelmed by excessive production of free radicals and reactive metabolites, the antioxidant defense system counter attack those radicals by neutralization process. However, when the antioxidant defense system itself is overwhelmed by many biological and environmental factors, more reactive radicals may be produced. This will cause damage to DNA and cellular tissues, leading to aging and diseases (Rahman *et al.*, 2012). This state explains the term of "oxidative stress" and its role of contribution towards development of many diseases.

Since oxidative stress can be encountered by antioxidant activities, it shows the importance of antioxidants in maintaining the health of human's body. Antioxidant is a substance that when present in low concentration, may prevent oxidation and thus, lowering the risk of cells damage and aging (Young & Woodside, 2001). Antioxidants will act by accepting or donating electron, resulting in either the free radicals being neutralized and destroyed, or becoming less reactive (Lu *et al.*, 2010). Antioxidants come from various sources; fruits, plants, whole grains and nuts.

Nowadays, medicinal plants are in focus as these plants are known to be the best source of antioxidants. Up to 80% of people around the world prefer using medicinal plants to cure various diseases as these plants are known to be antioxidative and antimicrobial (Ling *et al.*, 2011). The medicinal property of these medicinal plants lies in the presence of bioactive compounds known as phytochemicals. Phytochemicals are chemicals produced by plants which work effectively along

with nutrients and fibres in body protection mechanism (Krishnaiah *et al.*, 2009). Phytochemicals can be categorized into few; flavonoids, steroids, alkaloids, phenolic compounds, etc. It has also been reported that these bioactive compounds are involved in haemolytic and foam foaming activities (Santhi & Sengottuvel, 2016).

There are more than 250000 species of higher medicinal plants in Malaysia and yet, only around 15% of these plants have been studied (Ling *et al.*, 2011). Hence, we focus on *Lygodium circinnatum*, also known as the climbing fern in the family of Lygodiaceae. This plant is present mostly in countries with hot and humid temperatures. It is found in the wild and commonly used as medicine and food (Ginco *et al.*, 2016). This study was done to assess the presence of bioactive compounds and the antioxidant activity of aqueous extract of *L. circinnatum*. The output of this study may add values towards the medicinal property of the plant, which more investigation can be done in the future studies.

METHODOLOGY

Phytochemical Screening

Phytochemical screening tests were done following the standard methods (Azalina Farina & Iqbal, 2013).

Sample Collection

The plant chosen in this study is *Lygodium circinnatum* which is locally known in Sabah. The sample was collected at nearby hill to Odeck, Universiti Malaysia Sabah (UMS), Sabah, Malaysia. Once collected, the plant sample was washed under running tap water and dried at room temperature for 2 weeks followed by grinding process using a blender.

Aqueous Extraction

The sample was extracted using aqueous method according to (Azalina Farina & Iqbal, 2013). 100 g of sample was dissolved in a beaker of 1 Liter of boiling distilled water. After 10 min, the beaker was placed on a bench and cooled at room temperature for an hour. The aqueous extract was filtered using Whatman No. 1 filter paper. Then, the crude extract obtained was transferred into falcon tubes, kept at -80°C for 24 hr, then covered with parafilm with holes and stored back at -80°C until further lyophilisation using a freeze drier.

Total Phenolic Content

Total phenolic content was assessed using Folin-Ciocalteu method (Mitra & Uddin, 2014) with slight modification. 0.5 mL of Folin-Ciocalteu reagent which was prepared by ratio 1:10, was mixed with 0.5 mL plant extract and left for 5 min in the dark. 2 mL of sodium carbonate (60g/L) was then added and incubated for 30 min in the dark. The absorbance was measured at 765 nm and gallic acid was used as standard. The concentrations of the standard were set at 10, 20, 40, 80 and 100 µg/ml.

Total Flavonoid Content

Total flavonoid content was measured using aluminium chloride method (Rebaya *et al.*, 2014) with slight modification. 0.25 mL plant extract was mixed with 0.075 mL sodium nitrate (5%) and left in the dark for 6 min. Then, 0.15 mL of aluminium chloride (10%) was added, mixed and left for another 5 min. 0.75 mL NaOH was added followed by distilled water. The absorbance was measured at 510 nm. Catechin was used as the standard. The standard concentrations used were 10, 80, 100, 300 and 500 µg/ml.

Determination of Radical Scavenging Activity

Radical scavenging activity was done using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) method following (Azalina Farina & Iqbal, 2013) with slight modification. Various concentrations of plant extract and ascorbic acid as standard were prepared (20, 80, 160, 320, 640 µg/ml). Briefly, 3 mL DPPH in ethanol was mixed with 0.3 mL plant extract and kept in the dark for 30 min. The absorbance was measured at 517 nm. The radical scavenging activity was calculated using the following formula:

$$\% \text{ DPPH radical scavenging} = \frac{[(\text{Control absorbance} - \text{Sample absorbance}) / \text{Control absorbance}] \times 100}{1}$$

RESULT AND DISCUSSION

Phytochemicals Screening Tests

From the analysis, *L. circinnatum* has revealed the presence of the following; phenolics, flavonoids, tannins, alkaloids and glycosides. Two compounds which are saponins and terpenoids were found to be absent. Polyphenols including flavonoids are found to be abundant in plants and their ability as good and safe antioxidants often and currently still being studied.

Total Phenolics and Flavonoids Content Determination

The TPC and TFC of aqueous extract of *L. circinnatum* are presented in Table 1.

Table 1. Total phenolics and flavonoids of aqueous extract of *Lygodium circinnatum*.

Aqueous Extract of <i>L. circinnatum</i>	
Total Phenolics Content (mg GAE/g)	Total Flavonoids Content (mg CE/g)
31.84 ± 0.24	63.5 ± 1.67

Studies has reported that phenolics are the most abundant compounds in plant kingdom and its phytoconstituent are divided into three groups; flavonoids, polyphenols and phenolic acids. The antioxidants properties of phenolics determine their ability to scavenge free radicals (Saxena *et al.*, 2013). As shown in Table 1, the total phenolic and flavonoid content in aqueous extract of *L. circinnatum* are 31.84 ± 0.24 mg GAE/g and 63.5 ± 1.67 mg CE/g respectively. It was unusually yielded in higher flavonoids content than phenolics. Folin-Cioacalteau reagent consists of phosphotungstic-phosphomolybdenum complex which reacts with phenolics compound to form blue chromophore (Blainski *et al.*, 2013). The phenolics compound will react to FC reagent differently, depending on the number of the phenolics group (Othman *et al.*, 2014). This reagent also reacts with other compounds beside phenolics as reported by (Everette *et al.*, 2010). It is possible that the reaction of Folin Cioacalteau reagent with the compounds has not reached the endpoint. A study done by Jeetendra & Manish (2011), the phenolics content of the aqueous extract of *L. flexuosum* yielded only around 4.38 mg/GAE g.

Reportedly, gallic acid has antimicrobial properties against pathogens and this statement is supported by a study of Karamac *et al.* (2006) on its antimicrobial property. Synthetic derivatives of gallic acid has also been reportedly to possess anticancer, antioxidants, free radicals scavenger, neuroprotective effect properties and also involved in apoptosis process of cancer cells (Nayeem *et*

al., 2016). Barbalho *et al.* (2012) has reported that gallic acid, catechin and epicatechin inhibit the activity of pancreatic cholesterol esterase which directly decreases the level of cholesterol. Catechins are also been used to prevent diabetes type 2 and obesity.

DPPH Antioxidant Activity

DPPH assay is a method focusing on the reduction of stable DPPH free radical. DPPH free radical is reduced to DPPHH after being paired off with an antioxidant (hydrogen donor), causing the absorbance to decrease and the purple colour (maximum absorption at 517 nm) to decolourize (Shekhar & Anju, 2014). The more decolourization (yellow colour) occurs, the more scavenging activity likely to occur.

As can be seen in Figure 1, radical scavenging activity in ascorbic acid has reached its maximum value (25.36 + 0.32) % at 1 mg/ml. Whereas for plant extract, the activity was much slower as the concentration increased, the radical scavenging activity increased. The usage of different extraction solvent has the possibility affecting the antioxidant activity of the plant sample. Aqueous extract may exhibit lower activity compare to that in solvent extracts as DPPH is known to have a better solubility in organic solvents than in aqueous solution (Mamedov, 2012). Solvents extract will fully maximize the DPPH interaction with the antioxidants in the plant (Othman *et al.*, 2014). Obtaining higher yield of ascorbic acid can also be possible if compared with solvents extract of plant sample rather than aqueous. The IC₅₀ value found to be 39.43 and 143.76 µg/ml for ascorbic acid and aqueous extract respectively. Lower IC₅₀ signifies higher antioxidant activity.

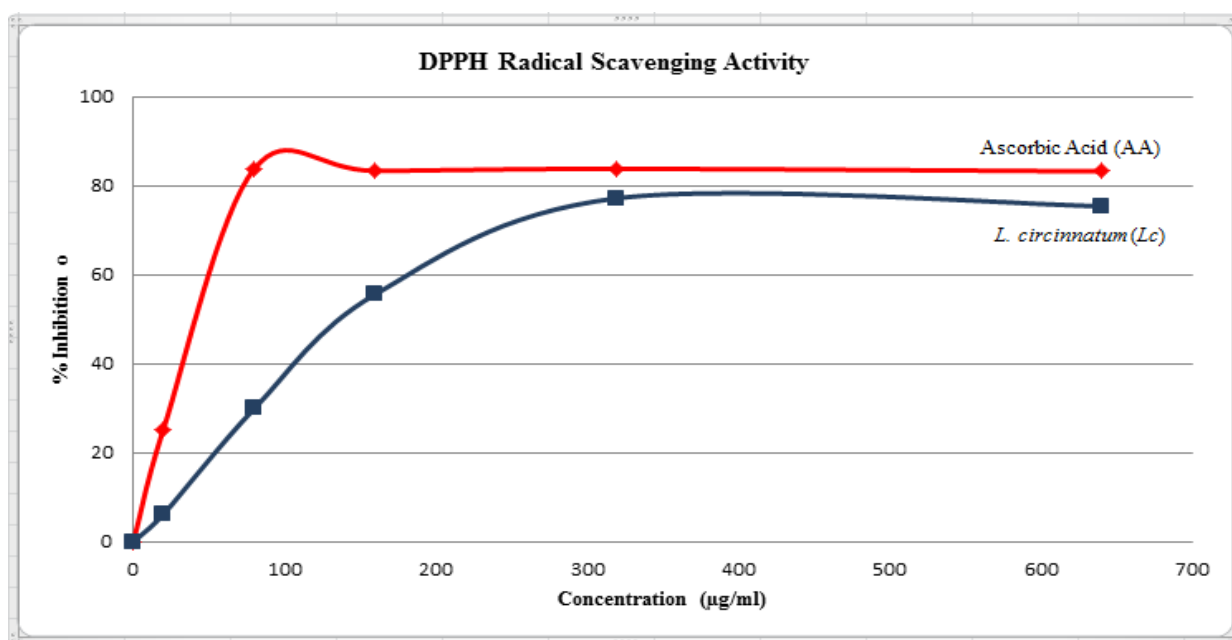


Figure 1. DPPH radical scavenging activities of aqueous extract of *L. circinnatum*, compared with ascorbic acid.

CONCLUSION

Proper extraction methods are the first important step of obtaining better yields of extracts with strong antioxidant activity. Further analysis and improvement of methods should be applied. The bioactive compounds and antioxidant activity of *L. circinnatum* may be considered in thorough future studies in order to determine the plant's medicinal potential.

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