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Evaluation of Total Phenolic Content and Antioxidant Activity in Aqueous and Ethanolic Extracts of *Leucosyke capitellata*

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ABSTRACT

Leucosyke capitellata is rich in tannins, steroids, and flavonoids and has been traditionally used in the Philippines and Sabah, Malaysia, for treating inflammation, hypertension, and diabetes. However, scientific research on *L. capitellata* remains limited. This study aims to investigate the total phenolic content (TPC) and antioxidant potential of the aqueous and ethanolic extracts from the leaves, roots, and stems of *L. capitellata*. The TPC was measured using the Folin-Ciocalteu method, and the antioxidant activity was assessed through DPPH scavenging. Among the extracts, the ethanolic root extract exhibited the highest TPC (135.655 ± 0.045 mg GAE/g). All extracts demonstrated significant antioxidant activity (IC_{50} < 50 µg/mL), with the leaves aqueous extract showing the highest antioxidant activity (IC_{50} = 5.274 µg/mL) despite having a relatively low TPC (37.737 ± 0.013 mg GAE/g). This discrepancy between TPC and antioxidant activity highlights the complex nature of plant extracts and suggests that factors beyond TPC contribute to their antioxidant mechanisms. These findings indicate that *L. capitellata* is a promising source of natural antioxidants.

Keywords: *Leucosyke capitellata; total phenolic content; antioxidant activity*

INTRODUCTION

Living organisms naturally produce reactive oxygen species (ROS) such as superoxide, hydroxyl, and peroxy radicals as part of their metabolism (Indirayati et al., 2020). However, excessive ROS production can lead to biomolecule oxidation and cellular damage, ultimately contributing to various disease such as diabetes, cancer, cirrhosis, obesity, and cardiovascular disorders (Jafri et al., 2023; Jafri et al., 2022). Several factors, including exposure to ultraviolet rays, cigarette smoke, environmental pollutants, and toxic chemicals, can trigger the overproduction of ROS (Jafri et al., 2023). Therefore, enzymatic antioxidant barriers like superoxide dismutase (SOD) can act as defense mechanisms to neutralize the harmful effects of free radicals, which can damage cells in living organisms (Aryal et al., 2019; Jafri et al., 2023). However, the levels of these endogenous defenders produced in the body may be insufficient, particularly during the generation of free radicals is heightened (Ulewicz-Magulska & Wesolowski, 2018). Therefore, dietary antioxidants may be necessary to maintain optimal cellular functions under increased oxidative stress (Indirayati et al., 2020).

There are two types of antioxidants: synthetic and natural. Synthetic antioxidants like propyl gallate, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are commonly used (Jafri et al., 2022; Ulewicz-Magulska & Wesolowski, 2018), but their safety has been questioned due to potential health risks and toxicity (Indirayati et al., 2020; Molole et al., 2022). On the other hand, natural antioxidants extracted from plants are highly recommended for food applications due to their safety, nutritional potential, and therapeutic effects (Indirayati et al., 2020; Ulewicz-Magulska & Wesolowski, 2018). Medicinal plants with documented traditional use in folk medicines for treating oxidative and related diseases such as hypertension and diabetes are promising sources for novel and effective antioxidants (Molole et al., 2022). For instance, natural flavonoids are secondary metabolites found in plants, characterized by an aromatic ring containing at least one hydroxyl group (Aryal et al., 2019). Flavonoids offer potential health advantages owing to their antioxidant properties, primarily driven by phenolic hydroxyl groups (Vo et al., 2019).

Leucosyke capitellata, a plant species belonging to the family Urticaceae, has been found to contain elevated levels of tannins, steroids, and flavonoids, along with a moderate quantity of saponins (Gawat & Eupeña, 2022). Moreover, this plant has a history where the roots decoction have been traditionally used among local healers in the Philippines for treating inflammation on wound (Gawat & Eupeña, 2022). Furthermore, in Sabah, Malaysia, the leaves decoction of *L. capitellata* has been utilized by locals to manage hypertension and diabetes (Fasihuddin & Holdsworth, 2003). Hence, the aim of this study is to quantify the total phenolic content (TPC) in the ethanolic and aqueous extracts of various parts of *L. capitellata*, including stems, roots, and leaves, using the Folin-Ciocalteu method with slight modification, as well as to determine their free radical scavenging activities using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method.

MATERIALS & METHODS

Plant Material

The plant samples utilized in this study were the stem, root, and leaf of *L. capitellata*. Distilled water and 70% ethanol were the solvents used in preparing the plant extracts. For the TPC assay, the standard sample, chemicals, and reagents included gallic acid (Sigma-Aldrich, US), Folin-Ciocalteu reagent (Sigma-Aldrich, US), and sodium carbonate (Bendosen, Malaysia). As for the DPPH radical scavenging assay, the standard sample and reagents consisted of ascorbic acid (R&M Chemicals, Malaysia) and DPPH reagent from Sigma-Aldrich, US.

Instrument

Rotary evaporator (Buchi R-210, Switzerland) and freeze dryer (Brand?) machine were used for the extract preparation. Furthermore, quartz cuvette and UV-VIS Spectrophotometer (Genesys 20, Thermo scientific, model 4001/4) were used for quantitative analysis for both TPC and DPPH radical scavenging assay.

Sample Preparation

The fresh *L. capitellata* roots, stems, and leaves were collected from Jalan Kiulu, Tamparuli, Sabah, Malaysia. The samples of roots, stems, and leaves were cleaned and sent to the Herbarium of Institute for Tropical Biology and Conservation (ITBC), University Malaysia Sabah (UMS) for taxonomy and morphological confirmation with the assistance of botanist Mr. Johny Gilsil from the institute. After collection, the samples were cut into pieces and dried in an oven at 40°C. The dried samples were then ground into powder, stored in a zip lock bag, and preserved at 4°C until further solvent extraction.

Preparation of Aqueous and Ethanolic Extract

The oven-dried powdered plant samples underwent extraction using distilled water and 70% ethanol via sonication. The sample-to-solvent ratio was 1:20 (w/v), and they were sonicated for 25 minutes at room temperature. The extraction method was adapted from Ranjha et al. (2021) with slight modification. After extraction, different steps were followed for each solvent. For the aqueous extract, the filtrate was transferred to a 50 mL falcon tube and frozen at -80°C for two days before being placed in a freeze dryer to obtain the raw aqueous extract. The process in the freeze dryer took about three days, and the resulting concentrated extracts were stored at -80°C for further analysis.

For the ethanolic extract, after filtration, the resulting filtrate was concentrated under reduced pressure for one to two hours at 60°C using a rotary evaporator (Buchi R-210, Switzerland). The resulting filtrate was then frozen at -80°C for two days before being placed in the freeze dryer to remove the 30% aqueous in the 70% ethanol solvent. This process also took about three days. Afterward, the extracts were stored at -80°C for further analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Kupina et al., 2018) with slight modification. Solutions of 1 mg/mL of both sample and gallic acid were prepared, with concentrations ranging from 50 to 250 µg/µL. The solvent used for gallic acid was methanol, while the solvent for plant extracts varied depending on their extraction method. Each concentration mixture was prepared with a final volume of 200 µL. After preparing the mixtures, 200 µL of each was dispersed into test tubes. Then, 1.5 mL of Folin-Ciocalteu reagent (diluted 1:9 with distilled water) was added to the test tubes, followed by incubation for 5 minutes at room temperature. Subsequently, 1.5 mL of sodium carbonate solution (0.06 g/mL) was added to each tube, and the mixtures were allowed to stand for 90 minutes at room temperature. The TPC was measured in a quartz cuvette using a spectrophotometer (Genesys 20, Thermo Scientific, model 4001/4) at 750 nm. A standard calibration curve of the gallic acid (standard sample) was prepared using concentrations of 0.05, 0.10, 0.15, 0.20, and 0.25 mg/mL of gallic acid. Finally, the TPC was expressed in milligrams of gallic acid equivalents per gram of plant extracts (mg GAE/g) and were calculated as follows:

$$TPC = (C \times V)/M$$

Where, C is the concentration of gallic acid determined from the standard curve (mg/mL), V is the volume of the extract (mL), and M is the mass of the plant extract (g). All samples were done in triplicate.

Determination of Antioxidant DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the extracts was assessed following the method outlined by Brand-Williams and Berset (1995) and Indirayati et al. (2020) with a slight modification. To prepare the stock solution of DPPH reagent, a concentration of 300 µM was used. The extract sample and ascorbic acid (standard) were prepared at five concentrations (5, 10, 20, 40, 80 µg/mL), with a stock concentration of 1 mg/mL for both. Each concentration mixture was prepared with a final volume of 10 mL. After preparing the mixtures, 2.5 mL of each was dispersed in triplicate, followed by adding 1 mL of

DPPH solution into the sample. The mixture was then resuspended and allowed to stand for 30 minutes. The entire procedure was conducted in the dark. The absorbance of the mixture was measured using a quartz cuvette by using UV-VIS spectrophotometer (Genesys 20, Thermo Scientific, model 4001/4) at 517 nm. The percentage activity of the samples to scavenge the DPPH radical was calculated using the formula:

$$\text{Inhibition (\%)} = [(A_B - A_S)] / A_B] \times 100$$

Where A_B is the absorption of blank samples (mixture of solvent with DPPH) and A_S is the absorption of extract/standard solution. The assays were carried out in triplicate. The Inhibitory Concentration (IC_{50}) value, which represents the concentration of extract required to inhibit 50% of DPPH activity, was determined using regression analysis from a graph plotting of scavenging activity of sample/standard against concentration of sample/standard where the equation from the graph will be generated as the relationship between the concentration of the sample/standard and the scavenging activity. IC_{50} value was then estimated using the linear regression equation as follows:

$$Y = ax + b,$$
$$IC_{50} = (50 - b)/a$$

Where "a" represents the slope of the line, and "b" represents the y-intercept.

RESULTS & DISCUSSION

Total phenolic content (TPC)

The total phenolic content (TPC) in both aqueous and ethanolic extracts of *L. capitellata* leaves, roots, and stems was determined using the Folin-Ciocalteu method. The results for aqueous extracts were obtained from a calibration curve ($y = 6.4217x + 0.017$, $R^2 = 0.9971$) utilizing gallic acid concentrations ranging from 0.05 to 0.25 µg/mL. Similarly, for ethanolic extracts, the results were obtained from a calibration curve ($y = 5.1771x + 0.0027$, $R^2 = 0.9987$) of gallic acid. Both measurements were expressed in gallic acid equivalents (GAE) per gram of dry extract weight.

Table 1 Total phenolic content of the aqueous and ethanolic extracts of *L. capitellata* leaves, roots, and stems.

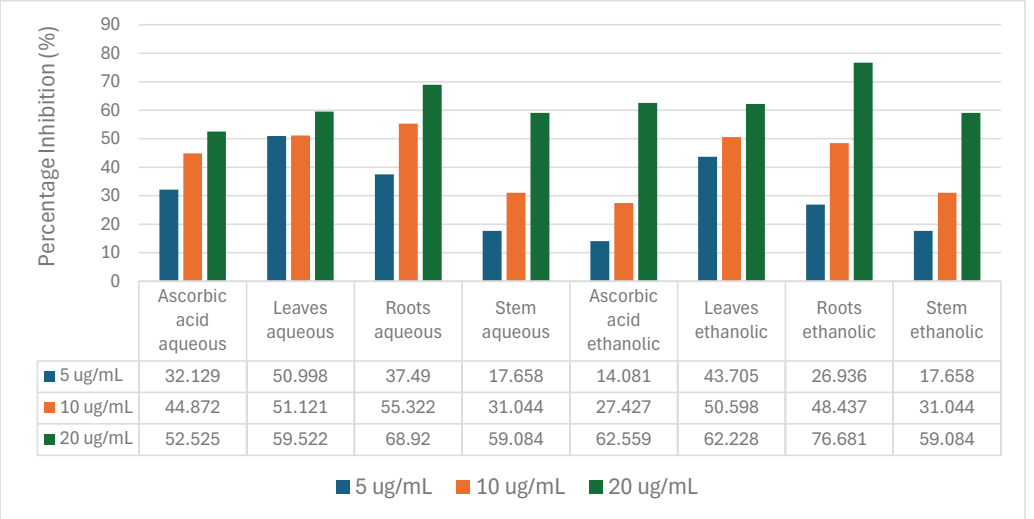
Leucosyke capitellata	Total phenolic content (mg GAE/g)	
	Aqueous extracts	Ethanolic extracts
Leaf	37.737 ± 0.013 [b]	73.657 ± 0.006 [a,b,c]
Root	117.563 ± 0.008 [a]	135.655 ± 0.045 [b]
Stem	34.762 ± 0.005 [a,c]	118.786 ± 0.004 [a,b,c]

All measurements were performed in triplicate, and the results are reported as the mean ± standard error mean (mean ± SEM). Different superscript letters indicate significant differences at $p < 0.05$, as analyzed by Tukey's multiple comparison test.

According to the findings in Table 1, the TPC in the aqueous extracts ranged from 34.762 mg GAE/g to 117.563 mg GAE/g, while for ethanolic extracts, it ranged from 73.657 mg GAE/g to 135.655 mg GAE/g. These results indicate that the ethanolic extracts of *L. capitellata* exhibited higher TPC compared to the aqueous extracts. In particular, the ethanolic extracts of root (135.655 mg GAE/g ± 0.045) showed the highest TPC, followed by stem ethanolic extracts (118.786 mg GAE/g ± 0.004) and leaf ethanolic extracts (73.657 mg GAE/g ± 0.006). The lowest TPC was observed in the aqueous extracts of stem (34.762 mg GAE/g ± 0.005). The extraction methods and solvents are responsible for dissolving the natural compounds present in the plants (Siddhuraju & Becker, 2003). Additionally, plant constituents can exhibit either polar or non-polar characteristics (Aryal et al., 2019). Phenolic compounds, containing hydroxyl groups which have higher solubility in polar organic solvents (Wang & Weller, 2006). Therefore, aqueous and 70% ethanol were chosen as the extracting solvents.

Antioxidant DPPH Radical Scavenging Assay

The DPPH scavenging assay operates on the principle of antioxidants interacting with DPPH, a free radical, through either electron transfer or hydrogen radical transfer. This interaction results in the neutralization of the DPPH radical (Indirayati et al., 2020). The quantification of DPPH scavenging was conducted after a 30 minute incubation period to allow for the reaction between DPPH as a free radical and the samples (Indirayati et al., 2020). The percentage inhibition (PI%) activity was presented in Figure 1, where all the extracts showed concentration-dependent increases in radical scavenging capacity.



	Ascorbic acid aqueous	Leaves aqueous	Roots aqueous	Stem aqueous	Ascorbic acid ethanolic	Leaves ethanolic	Roots ethanolic	Stem ethanolic
5 ug/mL	32.129	50.998	37.49	17.658	14.081	43.705	26.936	17.658
10 ug/mL	44.872	51.121	55.322	31.044	27.427	50.598	48.437	31.044
20 ug/mL	52.525	59.522	68.92	59.084	62.559	62.228	76.681	59.084

Figure 1 Comparison of percentage inhibition activity of ascorbic acid and L. capitellata leaves, roots, and stems aqueous and ethanolic extracts.

From Table 2, the highest DPPH radical scavenging potency with a minimum IC_{50} value was observed in the leaves aqueous extract *of L. capitellata* (5.274 µg/mL), followed by the aqueous extract of root (9.702 µg/mL), the ethanolic extract of leaf (9.889 µg/mL), the ethanolic extract of root (11.456 µg/mL), the ethanolic extract of stem (19.588 µg/mL), and finally the aqueous extract of stem (40.859 µg/mL).

Table 2 Comparison of antioxidant activity of the aqueous and ethanolic extracts of *L. capitellata* leafs, roots, and stems with ascorbic acid. The results are reported as the mean ± standard error mean (mean ± SEM).

Sample	IC_{50} value (µg/mL)	
	Aqueous extract IC_{50}	Ethanolic extract IC_{50}
Ascorbic acid	5.337 ± 0.006	4.885 ± 0.021
Leaf	5.274 ± 0.019	9.889 ± 0.025
Root	9.702 ± 0.006	11.456 ± 0.006
Stem	40.859 ± 0.003	19.588 ± 0.015

All data were compared with the IC_{50} value of standard ascorbic acid for both aqueous and ethanolic extracts. The utilization of IC_{50} in biological assays is extensively employed in pharmacological investigations (Benjamin et al., 2024). The classification of antioxidant activity based on IC_{50} is divided into five categories: highly active (<50 µg/mL), active (50 – 100 µg/mL), moderate (101 – 250 µg/mL), weak (250 – 500 µg/mL), and inactive (>500 µg/mL) (Indirayati et al., 2020).

The present study also reveals that both aqueous and ethanolic extracts of *L. capitellata* leaves, roots, and stems exhibit potent antioxidant properties. Remarkably, the aqueous extracts of the leaf show the highest antioxidant activity, with an IC_{50} value of 5.274 µg/mL ± 0.019, surpassing even the ascorbic acid standard (5.337 µg/mL ± 0.006). Despite this impressive antioxidant activity, the aqueous leaf extracts have one of the lowest total phenolic contents (37.737 ± 0.013 mg GAE/g). This paradox underscores the complexity of plant extracts and their antioxidant mechanisms, suggesting that factors beyond total phenolic content, such as other bioactive compounds, likely contribute to their observed antioxidant effects.

CONCLUSION

The total phenolic content (TPC) and antioxidant potential of the aqueous and ethanolic extracts from the leaves, roots, and stems of *L. capitellata* were investigated. The highest TPC was observed from root ethanolic extracts (135.655 mg GAE/g ± 0.045), while the lowest TPC was found from stem aqueous extract (34.762 mg GAE/g ± 0.005). All extracts in this study have a highly active antioxidant activity (IC_{50} <50 ug/mL). However, extracts that possess the highest antioxidant properties, as indicated by their DPPH scavenging activity, are from the leaf aqueous extract with the IC_{50} value of 5.274 ug/mL ± 0.019 and the lowest antioxidant properties was observed from the stem aqueous extract with the IC_{50} value of 40.859 µg/mL ± 0.003. Notably, the leaf aqueous extract had the second

lowest TPC (37.737 mg GAE/g ± 0.013). The discrepancy between TPC and antioxidant activity underscores the complexity of plant extracts and their antioxidant mechanisms, suggesting that factors beyond TPC may contribute to their antioxidant effects. A deeper investigation into the phenolic compositions and antioxidant properties is crucial for a thorough understanding of a plant extracts' antioxidant capacity.

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