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Research Article

Phytochemical Analysis, Anti-Inflammatory, and Antioxidant Activity of Selected Medicinal Plants in Mandiangin Rainforest in South Kalimantan, Indonesia

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ABSTRACT

Mandiangin is one of the tropical rainforests in South Kalimantan, with highly diverse plants used for health and medicinal purposes. To date, scientific evidence on these plants' content and bioactivities remain lacking for further medical applications. In this perspective, this study aimed to determine the phytochemical contents of six selected plants, which include the Bilaran Kusan (*Passiflora foetida*), Sembilakan (*Caesalpinia* sp), Bamban Batu (*Donax cenniformis*), Kilayu (*Aglaia* sp), Ulur-Ulur (*Tetrastigma* sp), and Mali-Mali (*Leea indica*) from the Mandiangin rainforest, in addition to their antioxidant and anti-inflammatory activity. Results revealed that the *Leea indica* extracts exhibited the highest flavonoid content (70.892 ± 0.34 mg/ml QE), while the highest tannin (2.101 ± 0.02 mg/ml GAE) and alkaloid contents (25.30 ± 0.71 %) were recorded for the *Aglaia* sp species. Meanwhile, *Passiflora foetida* showed the highest saponin content at (31.78 ± 2.97 %). Based on the study findings, the extracts of all six plants from the Mandiangin rainforest possessed medically valuable phytochemical constituents, as seen from their appreciable antioxidant and anti-inflammatory activity. Of all these plants, *P. foetida* and *L. indica* demonstrated the highest antioxidant activity, whereas the highest anti-inflammatory activity was noted for the P. *foetida*, Aglaia- and Caesalpinia species.

Keywords: Anti-inflammatory activity, Antioxidant activity, Medicinal plants

Introduction

Oxidative stress and inflammation are two strongly correlating issues that affect human health. This is because oxidative stress leads to inflammation, which then triggers oxidative stress. As a matter of fact, the two conditions are the cause for various other diseases, *viz*. infectious diseases, diabetes mellitus, and coronary heart disease, among others [1]. Fortunately, available treatments involving antioxidants may prove helpful in managing or averting the above-said diseases. The literature has shown that a dietary intake of antioxidant-rich, plant-based foods could effectively lower the risks of diseases in humans.

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Such an outcome explains the growing interest in naturally-derived substances or those of plant origin, showing high antioxidant activity that could safeguard humans against cellular-level damage due to oxidative stress [2].

Mandiangin is one of the tropical rainforest areas in south Kalimantan, rich in medicinal plants, such as *Passiflora foetida*, *Donax cenniformis*, *Aglaia sp*, and *Leea indica*. Results of empirical studies have shown that *P. foetida, Caesalpinia sp,* and *D. cenniformis* extracts are employed as traditional cough medicine. In contrast, the *Aglaia species* extract has therapeutic uses for treating chickenpox and herpes, and the *Tetrastigma species* extract is a traditional remedy for hemorrhoids [3,4]. In fact, these curative benefits of *P. foetida, D. cenniformis, Caesalpinia species, Aglaia species, Tetrastigma sp,* and *L. indica* seen above could be associated with their extracts containing various phytochemical compounds comprising flavonoids, alkaloids, and steroids. [3,4].

Despite their popularity as traditional remedies, there is little literature regarding the effects and the levels of the various phytochemicals in the above-said plants, thereby warranting further research by the scientific community. *Ipso facto*, this research aimed to determine the levels of flavonoids, tannin, alkaloid, and saponin, as well as determine the antioxidant capacity and anti-inflammatory activity of extracts from six selected plants from the Mandiangin rainforest in South Kalimantan, Indonesia. The six plants selected in this study are those commonly used by the locals to treat several diseases. Table 1 enlists their local- and scientific names, parts of the plants used, and their bioactivities [3].

Material and Methods

Plants Samples and Extraction

Stems of *P. foetida*, *Caesalpinia species*, *D. cenniformis*, and *Tetrastigma species*, leaves of *Aglaia species*, and the *L. indica* fruits were collected from the Mandiangin rainforest in South Kalimantan in November 2019. A taxonomist then confirmed the plants' identities from the Faculty of Forestry, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia. Briefly, the plant's stems, leaves, and fruits were rinsed with fresh water and brushed clean to remove dirt. The samples were dried in an oven at 50°C for 96 hours. Then, the plant materials were macerated in methanol (70% w/v), 400 mL, in which each plant sample was soaked in the solvent for four days. The methanol was decanted, and the plant materials were filtered. The filtrate was collected and concentrated on a rotary evaporator at 40°C.

Estimation of Flavonoid Content

The total flavonoid content in each sample was estimated according to a method by Chang [5,6] with some modifications. Briefly, 0.5 mL of the sample was diluted in distilled water (2 mL), followed by the addition of sodium nitrite (150 µL). After 6 min, solutions of aluminum chloride (150 µL) and sodium hydroxide (2 mL) were added to the mixture and stirred. Finally, the volume of each mixture was made up to 5 mL by adding distilled water and left to stand for 15 min. The absorbance of each solution was read on UV-Vis spectroscopy (UV VIS Spectrophotometer Labo-7809) at 520 nm, using quercetin as the standard. The concentration of the flavonoid was then expressed as mg/mL of quercetin.

Estimation of Tannin Content

In this study, the tannin content was spectrophotometrically estimated by diluting the sample (1 gram) in distilled water (50 mL). Next, an aliquot of the diluted sample (0.05 mL) was withdrawn and transferred to a test tube containing ferric chloride solution (0.4 mL dissolved in 0.1 M hydroxy chloride), followed by the addition of potassium hexacyanoferrate solution (0.4 mL, 0.8 mM). Distilled water was added to the solution to make up a final volume of 10 mL. The solution was allowed to stand for 7 min before the absorbance by a spectrophotometer UV-Vis (UV VIS Spectrophotometer Labo-7809) at 700 nm [7]. The concentration of the tannin was expressed as mg/ml GAE.

Estimation of Alkaloid Content

Alkaloids were determined using the Harborne method [5]. Each sample (10 grams) was transferred to a 250 mL glass beaker containing acetic acid in ethanol (200 mL, of 10%), and the beaker was closed and allowed to stand for 4 hours at room temperature. The sample was filtered, and 25% of the extract was evaporated in a water bath. Ammonium hydroxide was added dropwise to the extract and then precipitated. Next, the precipitate was rinsed with dilute ammonium hydroxide solu-

tion before filtering. The remaining residue represents the alkaloid content, expressed in percent for every 10 grams of the sample.

Estimation of Saponin Content

Saponin was determined using the method proposed by Obadoni and Ochuko [8] with some modifications. Each sample was ground, weighed to 10 grams before transferring into a glass beaker. Then, ethanol (200 mL, 20% w/v) was added, and the mixture was heated in a water bath at 55 °C for 4 h. The mixture was filtered and the residue was re-extracted with ethanol (200 mL, 20% w/v). Each extract was reheated in a water bath at 90°C to reduce its volume to 40 mL. The concentrated extract was transferred to a separatory funnel, and the aqueous layer was run off. Next, n-butanol (60 mL) was added to the extracts and shaken. The mixture was then rinsed with NaCl solution (5%, X mL) and evaporated. The resultant mixture was dried until a constant weight was obtained. The saponin content was expressed in the percent of the 10-gr sample.

Hydrogen Peroxide Scavenging Activity

Each plant extract was examined for hydrogen peroxide scavenging activity using the Ruch et al. method [9]. Briefly, hydrogen peroxide solution (40 mM in phosphate buffer solution, pH 7.4) was prepared. Then, the plant extract (0.25 mL) was added to the prepared hydrogen peroxide solution (0.6 mL) before adding distilled water (1 mL). The mixture was left to stand for 10 min to form the test solution (A1). Meanwhile, the blank solution (A0) was prepared by mixing the above-said hydrogen peroxide solution (0.6 mL) with distilled water (1.25 mL). Both A1 and A0 solutions were measured on a spectrophotometer at 230 nm, and the percentage of hydrogen scavenging activity was estimated using the following Equation 1:

$$\text{H2O2 scavenging activity} = \frac{(\text{A0} - \text{A1})}{\text{A0}} \times 100 \quad (1)$$

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of each extract was determined by the method of Chung et al. [10-11] with minor modifications. Hydrogen peroxide undergoes a series of reactions called the Fenton reaction, which liberates hydroxyl radicals in the presence of iron. In this study, the test tubes for the Fenton reaction mixtures comprised 0.06 mL of 1 mM ferric chloride, 0.09 mL of 1 mM o-phenanthroline, 2.4 mL of 0.2 M phosphate buffer with 7.8 pH, 0.15 mL of 0.17 M hydrogen peroxide, and 1.5 mL of the plant extract. Each mixture's absorbance was read on a spectrophotometer at 560 nm (A0). The mixture was left to stand for 5 min before the absorbance was measured again to obtain A1. The following Equation 2 was used to estimate the percentage of hydroxyl radical scavenging activity:

$$\text{OH scavenging activity} = \frac{(\text{A0} - \text{A1})}{\text{A0}} \times 100\% \quad (2)$$

Chelating Effect of Metal Ions

The method developed by Dinis et al. was used to gauge the chelating effect of metal ions [12-13]. In this work, the test tube containing a mixture of the plant extract (0.25 mL), ferrous chloride solution (0.05 mL, 2 mM), *o*-phenanthroline (0.2 mL), and distilled water (1 mL) were mixed to form the A1 test solution. Meanwhile, the blank solution (A0) comprised a mixture of ferrous chloride, *o*-phenanthroline, and distilled water without the plant extract. Both mixtures were left to stand for 10 min, and their absorbances were measured on a spectrophotometer at 562 nm. The percentage of the chelating effect of metal ions was estimated using the following Equation 3:

$$\text{Chelating effect of metal ions activity} = \frac{(\text{A0} - \text{A1})}{\text{A0}} \times 100\% \quad (3)$$

Evaluation of Protein Denaturation Inhibition

The anti-inflammatory activity of each plant extract was gauged on its ability to inhibit protein denaturation. In this assessment, three solutions, namely, the test- (AT), control- (AC), and standard (AS) solutions, were prepared in test tubes. The AT solution consisted of bovine serum albumin (0.45 mL, 5% w/v) and plant extract (0.05 mL), while the AC solution comprised a mixture of bovine serum albumin (0.45 mL, 5% w/v) and distilled water (0.05 mL). The AS solution was a mixture of bovine serum albumin (0.45 mL, 5% w/v) and diclofenac sodium (0.05 mL). Then, HCl (1 N) was added to each solution and incubated for 20 min at 37°C before adjusting their pH to 6.3. Each solution was incubated for a further 30 min

at 57°C and then cooled to room temperature before adding the phosphate buffer (2.5 mL, pH 7.4). The absorbance of each solution was read on a spectrophotometer at 416 nm [14], and Equation 4 was used to estimate the inhibition of protein denaturation (%):

$$\%\ \text{of protein denaturation inhibition} = \frac{(\text{AT} - \text{AC})}{\text{AC}} \times 100\% \quad (4)$$

Evaluation of Heat-Induced Haemolysis Inhibition

The inhibition of heat-induced hemolysis was the method used in this study to the measure anti-inflammatory activity of the plant extract. Briefly, the test solution (AT) consisted of plant extract (1 mL) and red blood cell (RBC) suspension (1 mL, 10%), while the control (AC) solution consisted of aspirin (1 mL, 5 g of aspirin dissolved in 100 mL of water) and also the RBC suspension. The mixtures were incubated (57°C, 30 min), cooled under running tap water, and then centrifuged (2500 rpm, 5 min). The absorbance of each mixture was measured at 560 nm [14], and the percentage inhibition of heat-induced haemolysis was calculated according to Equation 5:

$$\%\ \text{of heat induce haemolysis inhibition} = \frac{(\text{AT} - \text{AC})}{\text{AC}} \times 100\% \quad (5)$$

Evaluation of Hypotonicity-Induced Haemolysis Inhibition

This study tested the anti-inflammatory properties of each plant extract using the RBC induced by a hypotonic solution, according to a method described by Islam et al. [15] with minor modifications. An aliquot of the test solution (AT) comprising the plant extract (1 mL) was transferred to a test tube containing a mixture of phosphate buffer (1 mL, 10 mM, pH 7.4), RBC suspension (0.5 mL), and hypotonic solution (NaCl 0.4% w/v, 2 mL). The control solution (AC) consisted of diclofenac sodium solution (1 mL), phosphate buffer (pH 7.4), RBC suspension, and hypotonic solution with the same characteristics as the test solution. Each solution was incubated at 37°C for 30 min and then centrifuged (3000 rpm, 5 min). An aliquot of each solution was withdrawn, and the absorbance was read at 560 nm [15]. The inhibition of heat-induced haemolysis (%) was calculated using the following Equation 6:

$$\%\ \text{of hypotonicity induced haeomlysis inhibition} = \frac{(\text{AT} - \text{AC})}{\text{AC}} \times 100 \quad (6)$$

Statistical Analysis

The data presented in this study were the mean ± standard deviation of three independent replicates. The results were compared by one-way analysis of variance (ANOVA), and Tukey's test examined the significant differences among the means. All statistical analyses were performed using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) software. Differences among means at a 5% level ($P < 0.05$) were considered statistically significant.

Results and Discussion

Phytochemical Levels

The phytochemical content of *P. foetida, Caesalpinia species*, *D. cenniformis*, *Tetrastigma species* stems, *Aglaia species* leaves, and *L. indica* fruits were investigated in this study. The results are presented in Figures 1, 2, and 3, respectively.

Figure 1 shows that the *L. indica* extract exhibited the highest flavonoid level, followed by the *Aglaia species*, *D. cenniformis*, *Caesalpinia species*, *Tetrastigma species*, and *P. foetida*. Also, the total flavonoid contents of the *L. indica*, *Aglaia species*, and *D. cenniformis* methanolic extracts were significantly higher ($p<0.05$) than the *Caesalpinia species*, *Tetrastigma species*, and *P. foetida*.

Meanwhile, the tannin level is the highest for the *Aglaia species*, ensued by the *Caesalpinia species*, *D. cenniformis*, *L. indica*, *Tetrastigma species*, and *P. foetida* (Figure 2). The total tannin content in the methanolic extracts of *Aglaia species, Caesalpinia species*, *D. cenniformis*, and *L. indica* were also significantly higher ($p<0.05$) compared to extracts obtained from *Tetrastigma species* and *P. foetida*.

However, the saponin levels amongst the plants were ordered differently, where *P. foetida* was the highest, followed by *L. indica*, *Aglaia species*, *Caesalpinia species*, *Tetrastigma species*, and lastly, *D. cenniformis* (Figure 3). Also, significantly higher total saponin contents were observed for the methanolic extracts of *P. foetida* and *L. indica* ($p<0.05$) than those from *Aglaia* species,

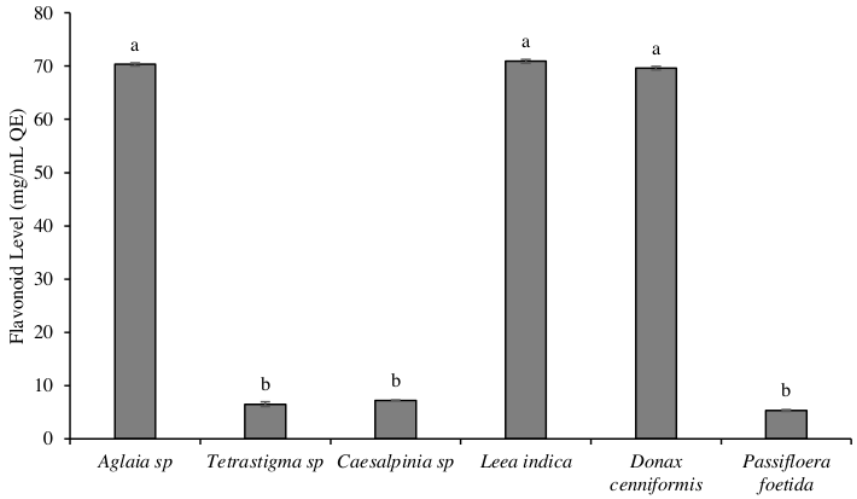

Figure 1. Flavonoid contents of the methanolic extracts from six plants collected from Mandiangin rainforest in South Kalimantan, Indonesia. Flavonoid content is expressed as mg of rutin-equivalents per g of dry extract. Results are means ± SE (n = 3). Different letters above the average bars denote significant differences at $p < 0.05$ - Tukey's test.

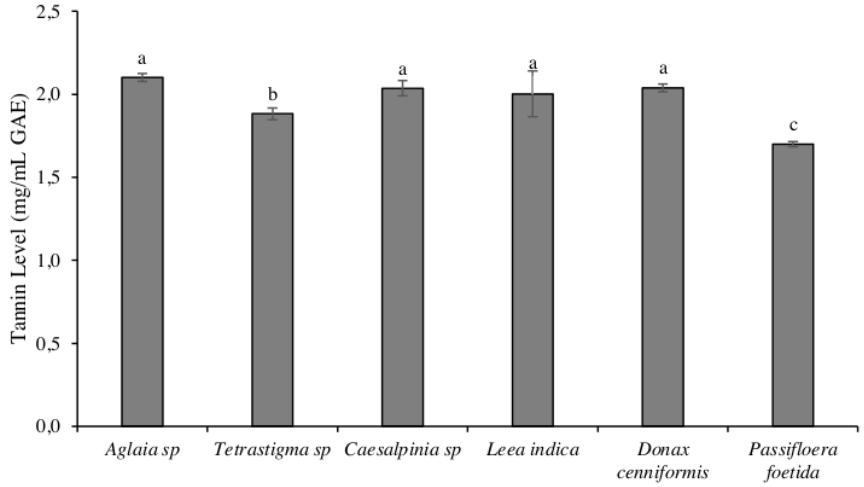

Figure 2. Tannin contents of the methanolic extracts from six plants collected from Mandiangin rainforest in South Kalimantan, Indonesia. Tannin content is expressed as mg of rutin-equivalents per g of dry extract. Results are means ± SE (n = 3). Different letters above the average bars denote significant differences at $p < 0.05$ - Tukey's test.

Caesalpinia species, *Tetrastigma* species, and *D. cenniformis*.

Finally, the alkaloid contents in the six plant species were identified in the following descending order: *Aglaia* species > *P. foetida, L. indica* > *Caesalpinia* species > *Tetrastigma* species > *D. cenniformis* (Figure 3). The total alkaloid content of the methanolic extract in the *Aglaia* species was also significantly higher ($p<0.05$) than other plants, except for extracts from *Caesalpinia* species and *P. foetida*. However, their total alkaloid

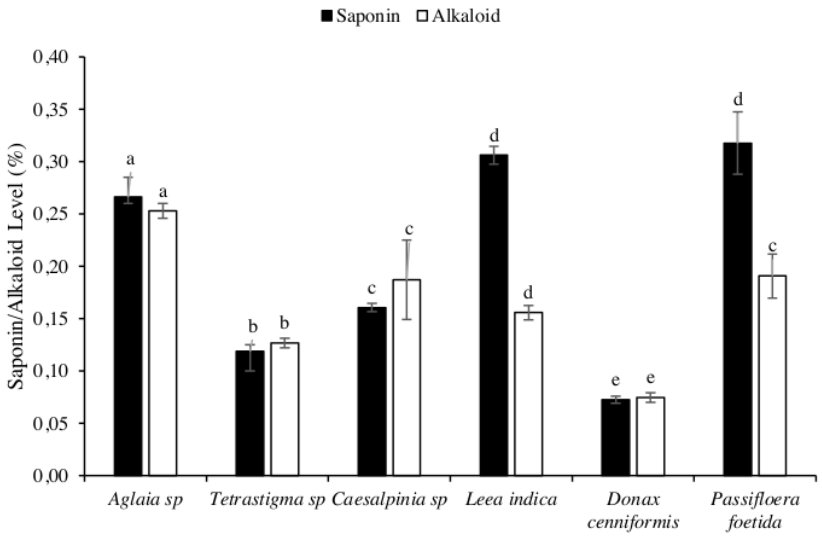

Figure 3. Saponin and alkaloid contents of the methanolic extracts from six plants collected from Mandiangin rainforest in South Kalimantan, Indonesia. Saponin and alkaloid content is expressed as % of 10-gram sample extract. Results are means ± SE (n = 3). Different letters above the average bars denote significant differences at $p < 0.05$ - Tukey's test.

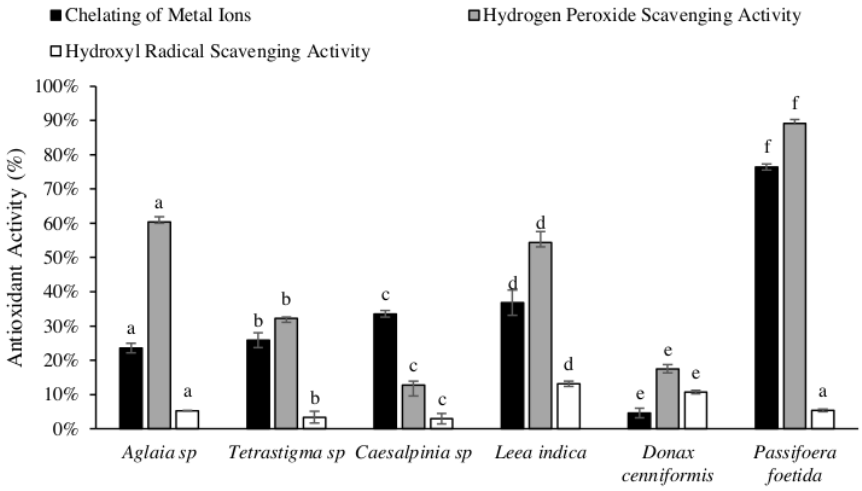

Figure 4. The antioxidant activity of the methanolic extracts from six plants collected from Mandiangin rainforest in South Kalimantan, Indonesia. The results are means ± SE (n = 3). Different letters above the average bars denote significant differences at $p < 0.05$ - Tukey's test.

contents were found to be insignificantly different ($p>0.05$).

Notably, previous studies documented similar results for the extracts from the stems of *P. foetida*, *Caesalpinia* species, *D. cenniformis*, and *Tetrastigma* species, in addition to the *Aglaia* species leaves extract [16-20]. For the tests mentioned above, there is no data for the *L. indica* fruit except for leaves [21]. However, flavonoids have been re-

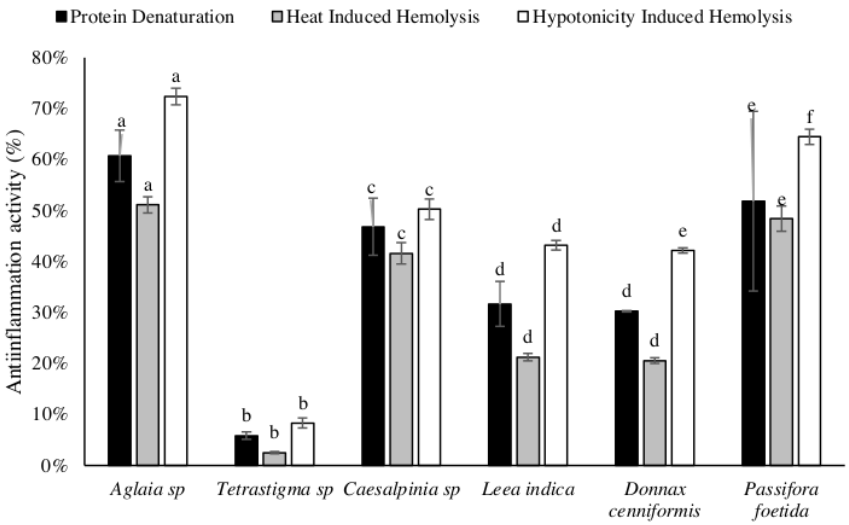

Figure 5. The anti-inflammation activity of the methanolic extracts from six plants collected from Mandiangin rainforest in South Kalimantan, Indonesia. The results are means ± SE (n = 3). Different letters above the average bars denote significant differences at $p < 0.05$ - Tukey's test.

ported in the stems of *P. foetida, Caesalpinia* species, *D. cenniformis,* and *Tetrastigma* species, alongside other compounds such as phenolics, tannins, alkaloids, saponins, and steroids, among others [16-19]. Likewise, several compounds comprising alkaloids, flavonoids, phenols, tannins, coumarins, quinones, fatty acids, and leucoanthocyanins were discovered in the leaf extracts of *Aglaia* species [20]. However, to the best of our knowledge, this is the first study investigating the phytochemical contents of *L. indica* fruits.

Antioxidant Activity

Results revealed that antioxidant activity existed in all six plants, specifically hydrogen peroxide- and hydroxyl radical scavenging activity and metal ions chelation. Notably, the methanolic extract of *P. foetida* was significantly the highest for hydrogen peroxide scavenging activity and metal ions chelation ($p<0.05$) (Figure 4). Meanwhile, the methanolic extract of *L. indica* exhibited the highest hydroxyl radical scavenging activity and was significantly higher ($p<0.05$) than other plant extracts.

It is a known fact that superoxide dismutase catalyses the oxidation reaction, which converts oxygen to superoxide radicals. Further catalysis by catalase and glutathione peroxidase converts the radical superoxide to hydrogen peroxide [16]. The above-said reactions are consequences of the Haber-Weiss and Fenton reactions, in which the former generates hydroxyl and hydroxide ions when H_2O_2 reacts with superoxide ions catalyzed by iron. Similarly, the Fenton reaction forms hydroxide- and hydroxyl radicals by a reaction between iron (II) and hydrogen peroxide. Thus, superoxide, hydrogen peroxide, and hydroxyl radical are examples of reactive oxygen species (ROS). These highly reactive compounds further interact with other macromolecules, such as lipids, glucose, and protein, to induce cell damage [17].

Nevertheless, antioxidants can inhibit the above-said chain reaction, which generates ROS. The mechanisms of ROS inhibition by antioxidants can occur through several pathways, including scavenging or by metal ions chelation. In fact, the extract of *P. foetida* has been documented to exhibit several types of antioxidant activities [21]. The plant's phytochemical compounds could also inhibit cytokine-related pro-inflammation, as well as the activity of kinase and phosphodiesterase enzymes which stimulate inflammatory responses [22,23].

Conclusion

The study findings demonstrated that all six plant extracts (*P. foetida*, *Caesalpinia* species, *D. cenniformis*, *Aglaia* species, *Tetrastigma* species,

and *L. indica*) possessed antioxidant and anti-inflammatory activities. In fact, their antioxidant and anti-inflammation activity might be due to several phytochemical constituents in the extracts, namely, flavonoids, tannins, alkaloids, and saponins. These compounds are also responsible for hydrogen peroxide- and hydroxyl radical scavenging activity, and metal ion chelation. In short, all extracts from the six plants collected from the Mandiangin tropical rainforests in South Kalimantan were shown to be promising sources of natural exogenous antioxidants and anti-inflammation agents. The plant extracts' therapeutic nature is attributable to their strong antioxidant and anti-inflammation activities. Therefore, further studies should isolate these beneficial phyto-compounds to determine their antioxidant potential. Most importantly, their antioxidant and anti-inflammation activities should be estimated from pure compounds or fractions of the extracts.

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