JTROLIS

PHYTOCHEMICAL ANALYSIS, ANTI-INFLAMMATORY, AND ANTIOXIDANT ACTIVITY OF SELECTED MEDICINAL PLANTS IN MANDIANGIN, SOUTH KALIMANTAN, INDONESIA

Reviewers' comment

This study highlighted the phytochemical analysis, anti-inflammatory, and antioxidant activity of selected medicinal plants in Mandiangin, South Kalimantan, Indonesia. However, major improvements need to be made for this manuscript to be published. I have provided numerous remarks on the text as it is often vague and needs further explanation or rephrasing.

Title

Revise the title. Make it short and concise.

Abstract

The result in the abstract is only descriptive.

The maximum number of keywords need to be checked with JTROLIS guideline.

Introduction

Table 1 from the results section can be included as part of the introduction, but not in results.

Materials and methods

The citation of the authors in the methods section should be cited soon after the name of the author, not at the end of the paragraph.

The part of the plants used in this study is not well explained.

The major flaw in the methodology is that it does not address the statistical analysis used in this study.

Some of the grammatical error, incomplete sentences, and rephrasing need to be done (refer to the main text file).

Results and discussions

The results and discussion is only superficial because there was only descriptive results without any statistical analysis. Thorough results and discussion need to be included. The results should be expressed as Mean +/- SD. It should then be discussed if there is any significant difference between the plants. It also needs to be discussed and supported with previous literatures (if any).

Study Limitations

The author should include if there is any limitation in the study.

2485 / **Nugroho et al.** / Phytochemical Analysis, Anti-Inflammatory, and Antioxidant Act **Library**

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Journal of Tropical Life Science

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Submission accepted.

Notifications

[JTLS] Editor Decision	2022-09-05 04:36 PM
[JTLS] Editor Decision	2022-09-13 04:49 PM
[JTLS] Editor Decision	2022-09-24 04:29 AM
[JTLS] Editor Decision	2022-11-24 03:06 AM

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[JTLS] Editor Decision

2022-09-05 04:36 PM

Iskandar Thalib:

We have reached a decision regarding your submission to Journal of Tropical Life Science, "PHYTOCHEMICAL ANALYSIS, ANTI-INFLAMMATORY, AND ANTIOXIDANT ACTIVITY OF SOME SELECTED MEDICINAL PLANTS IN TROPICAL RAIN FOREST OF MANDIANGIN, SOUTH KALIMANTAN, INDONESIA".

Our decision is to: Minor revision

Fahrul Huyop

Reviewer A:
Recommendation: Revisions Required

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14. Comments for the Authors :----------

Reviewer C:
Recommendation: Revisions Required

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14. Comments for the Authors :

After going through the submitted work, found that the present work was unique and has scientific significance. All the data results/manuscript formats are systematically arranged and presented within the manuscript. However, some rectifications are needed to fit into the journal standard guidelines. These are highlighted below in a point wise manner.
1. Result data were not sufficient in the abstract section. include all the finding data within the abstract

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Study Limitations

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Reviewer A:
Recommendation: Revisions Required, the AUTHOR FAILED TO ADDRESS ALL COMMENTS>

Reviewer B:
Recommendation: Resubmit for Review

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Notifications ×

[JTLS] Editor Decision

2022-09-24 04:29 AM

Dear Iskandar Thalib:

RE: #2485
I am willing to consider your article for publication provided:

a)that you send it in clean form after taking into consideration suggestions given by reviewers & put it into the Journal format, Figures/tables & References.
b) send for English editing services, to make sure the language is smooth understandable and easy to follow,

Thank you.

Fahrul Huyop

Journal of Tropical Life Science Universitas Brawijaya

Notifications ×

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Fahrul Huyop

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Article

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Contribution to the manuscript	:	Conceived the presented idea, verified the analytical methods, carried out the experiment, and supervised the findings of this work

Article

Phytochemical Analysis, Anti-Inflammatory, and Antioxidant Activity of Some Selected Medicinal Plants in Tropical Rain Forest of Mandiangin, South Kalimantan, Indonesia

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ABSTRACT

Mandiangin is one of the tropical rain forests which is located in South Kalimantan. There are many plants in this area that has been used in health and medicine. However, until now there is still lack of scientific evidence about the content and activities of these plants, so they can be used in the field of medicine. This study was designed to determine the phytochemicals content, antioxidant, and anti-inflammatory activity of 6 selected plants which were collected~~derives~~ from Mandiangin. These 6 plants including Bilaran Kusan (*Passiflora foetida*), Sembilikan (*Caesalpinia* sp), Bamban Batu (*Donax cenniformis*), Kilayu (*Aglaia* sp), Ulur-Ulur (*Tetrastigma* sp), and Mali-Mali (*Leea indica*). The results showed that the extracts of *Caesalpinia* sp has the highest flavonoid content, *Aglaia* sp has the highest tannin and alkaloid content, and *Passiflora foetida* has the highest saponin content. In conclusion, results of this study indicated that the 6 selected plants from Mandiangin possessed phytochemical constituents, antioxidant and anti-inflammatory activity. From all this plants, *Passiflora foetida* and *Leea indica* has the most antioxidant activity and Aglaia sp, *Passiflora foetida*, and *Caesalpinia* sp has the most anti-inflammatory activity.

Keywords: *anti-inflammatory activity, antioxidant activity, medicinal plants, Passifora foetida, Caesalpinia sp, Donax cenniformis, Aglaia sp, Tetrastigma sp*

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Introduction

Oxidative stress and inflammation are two things that have strong correlation. Oxidative stress can cause inflammation and inflammation also can trigger oxidative stress. These two conditions are known to be responsible for various diseases, including infectious diseases, diabetes mellitus, coronary heart disease, and others [1]. However, this process can be inhibited by antioxidants. Reports suggested that the dietary intake of antioxidant-rich plant source foods could decrease the incidence of human disease. Recently, there is a growing interest in substances from natural sources or plants exhibiting antioxidant properties that can be used to protect human beings from oxidative stress damage [2].

Mandiangin is one of tropical rain forest areas in south Kalimantan which has many medicinal plants, such as *Passiflora foetida*, *Donax cenniformis*, *Aglaia sp*, and *Leea indica*. Based on empirical studies, people have been used *Passiflora foetida, Caesalpinia sp,* and *Donax cenniformis* as traditional cough medicine. While *Aglaia sp* is often used as chicken pox and herpes therapy. Besides that, *Tetrastigma sp* is also used as traditional haemorrhoid medicine [3,4].

Result from previous study showed that *Passiflora foetida, Caesalpinia sp, Donax cenniformis, Aglaia sp, Tetrastigma sp,* and *Leea indica* contain flavonoid, alkaloid, and steroid qualitatively. The use of this plant in several diseases may be caused by the content of phytochemical compounds present in these plants [3,4]. However, these effects and the level of phytochemical content in all these plants needs further research. Therefore, this research will determine the level of flavonoid, tannin, alkaloid, and saponin, and antioxidant as well as anti-inflammatory activity of some selected plants from Mandiangin Tropical Rain Forest, South Kalimantan, Indonesia.

Material and Methods

Plants Samples and Extraction

Stems of *Passiflora foetida*, *Caesalpinia sp*, *Donax cenniformis*, and *Tetrastigma sp*, leafs of *Aglaia sp*, and fruits of *Leea indica* were collected from Mandiangin Tropical Rain Forest, South Kaliamntan, Indonesia, in November 2019. The identity of plants was confirmed Taxonomist from Forestry Faculty, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia. The different parts of all plants were washed with fresh water and brushed. The samples then dry and heated with oven at 50^0C for 96 hours. 400 ml of 70% methanol was~~70%~~ added to each sample and soaked for 4 days. Plant materials then removed from the solvents with filtration, and the filtrate was concentrated using a rotary evaporator.

Commented [A3]: Which part of the plants were used? Leaf, stem, root?

Estimation of Flavonoid Level

The total flavonoid content in the sample was estimated by the method of Chang with some modifications. A volume of 0.5 ml of the sample was diluted to 2 ml distilled water. 150 µl of sodium nitrite was added and after 6 min, 150 µl of aluminium chloride solution was added. 2 ml of sodium hydroxide was added and distilled water was added until the volume of the tube reaches 5 ml and kept for 15 min. The solution absorbance then measured with UV-Vis spectroscopy at 520 nm along with standard quercetin. The results expressed as mg/ml of quercetin [5,6].

Estimation of Tannin Level

Estimation of tannin level was determined using spectrophotometric method. 1 gram of sample and diluted with 50 ml of distilled water. 0.05 of diluted sample was taken and 0.4 ml of ferric chloride in 0.1 M hydroxy chloride and 0.4 ml of 0.8 mM potassium hexacyanoferrate were added. Distilled water is added to the solution until the volume of the solution becomes 10 ml. The solution was allowed to stand for 7 minutes and the absorbance was measured using a spectrophotometer at a wavelength of 700 nm [7].

Estimation of Alkaloid Level

Alkaloids were determined using Harborne method. 10 grams of the sample was put in a 250 mL glass beaker. 200 mL of 10% acetic acid in ethanol was added and the beaker was closed and allowed to stand for 4 hours. The sample was then filtered and a quarter of the extract was evaporated with a water bath. Ammonium hydroxide is added dropwise and then precipitated. The precipitate was then washed with dilute ammonium hydroxide and filtered. The remaining residue is an alkaloid. Alkaloid content is expressed in percent of 10-gr sample [5].

Estimation of Saponin Level

Saponin was determined using Obadoni and Ochuko methods with some modification. The samples were ground and weighed 10 gr, and put into glass beaker. 200 ml of 20% ethanol was added and heated in water bath for 4 h with temperature 55^{0}C. The mixture was filtered and the residue re-extracted with ethanol. The extracts heated again in water bath at temperature 90^{0}C and the volume was reduced to 40 ml. the concentrated then poured in a separatory funnel and the water layer was taken. 60 ml n-butanol added to the extracts. The mixture then washed with 5% NaCl and evaporated. The mixture was dried and a constant weight was obtained. Saponin content was expressed in percent of 10-gr sample [8].

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of the extract was determined by the method of Ruch et al. The hydrogen peroxide 40 mM in phosphate buffer solution was prepared. 0.25 ml of extract mixed with 0.6 ml of 40 mM hydrogen peroxide in phosphate buffer. 1 ml of distilled water was added to the mixture and let stands for 10 min. This solution was the test solution (A_1). For the blank solution (A_0), 0.6 ml of 40mM hydrogen peroxide in phosphate buffer mixed with 1.25 ml distilled water. Both A_1 and A_0 solution was measured using spectrophotometer at a wavelength 230 nm [9]. The percentage of hydrogen scavenging activity was calculated using equation:

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

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the extract was determined by the method of Chung et al. with slight modification. Hydrogen peroxide is able to undergo a set of reaction known as the Fenton reaction to release the hydroxyl radical in the presence of iron. The Fenton reaction mixture consisted 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. The absorbance of the mixture then measured using spectrophotometer at a wavelength 560 nm (A_0) and let it stands for 5 min. After 5 min, the absorbance was measured again with the same wavelength (A_1) [10-11]. The percentage of hydroxyl radical scavenging activity was calculated using equation:

$$\cdot OH\ scavenging\ activity = \frac{(A0 - A1)}{A0} \times 100$$

Chelating Effect of Metal Ions

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The chelating effect of metal ions was determined by the method of Dinis. 0.25 ml of plant extract mixed with 0.05 ml of 2 mM ferrous chloride, 0.2 ml o-phenanthroline, and 1 ml distilled water. This mixture was test solution (A_1). The blank solution (A_0) was the mixture of ferrous chloride, o-phenanthroline, and distilled water without plant extract. Both mixtures were stands in 10 min, and the absorbance was measured using spectrophotometer at a wavelength 562 nm [12-13]. The chelating effect of metal ions (%) was calculated using equation:

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

Evaluation of Protein Denaturation Inhibition

The inhibition of protein denaturation is one of the methods used to measure anti-inflammatory activity. There were three solutions made, including test (A_T), control (A_C), and standard (A_S) solution. The test solution consisted of 0.45 ml of 5% bovine serum albumin and 0.05 ml of plant extract. The control solution consisted of 0.45 ml of 5% bovine serum albumin and 0.05 ml of distilled water. The standard solution consisted of 0.45 ml of 5% bovine serum albumin and 0.05 ml of diclofenac sodium. 1 N HCl was added to each solution and incubated for 20 min at 37°C to adjust the pH to 6.3. Each solution then incubated again at 57°C for 30 min. All solution were cooled and then 2.5 ml of phosphate buffer was added. Furthermore, measured the absorbance of each solution using spectrophotometer at 416 nm [14]. The inhibition of protein denaturation (%) was calculated using equation:

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

Evaluation of Heat Induced Haemolysis Inhibition

The inhibition of heat induced haemolysis is also one of the methods used to measure anti-inflammatory activity. The test solution (A_T) consisted of 1 ml plant extract and 1 ml of 10% Red Blood Cell (RBC) suspension, while the control (A_C) solution consisted of 1 ml aspirin and also RBC suspension. All solution incubated at 57°C for 30 min. The solution then cooled under running tap water and centrifuged at 2500 rpm for 5 min. The supernatants were taken and the absorbance were measured at 560 nm [14]. The inhibition of heat induced haemolysis (%) was calculated using equation:

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

Evaluation of Hypotonicity Induced Haemolysis Inhibition

The anti-inflammatory effects of plant extract were investigated on haemolysis of RBC induced by a hypotonic solution and were evaluated using the described method with slight modification. The test solution (A_T) consisted of 1 ml of plant extract was taken and then 1 ml of 10 mM phosphate buffer, 0.5 ml of RBC suspension, and 2 ml of hypotonic solution (NaCl 0.4%) were added. The control solution (A_C) consisted of 1 ml of diclofenac sodium, phosphate buffer, RBC suspension, and hypotonic solution with the same characteristics as test solution. All solution incubated at 37°C for 30 min and centrifuged at 3000 rpm for 5 min. The supernatants were taken and the absorbance were measured at 560 nm [15]. The inhibition of heat induced haemolysis (%) was calculated using equation:

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

Result and Discussion

Phytochemical Levels

This study uses 6 plants commonly used by the local community for the treatment of several diseases. Descriptions of these 6 plants consisting of local names, scientific names, parts that have been used, and the medicinal activities are presented in table 1.

The results from table 1 shows that 6 plants have an effect on several diseases. *Passiflora foetida* can lower blood sugar levels and is beneficial in diabetic patients. *Caesalpinia sp* and *Donax cenniformis* are known to treat cough in acute respiratory infections. *Aglaia sp* is useful in chickenpox and herpes zoster. *Tetrastigma sp* can reduce gastrointestinal bleeding and treat haemorrhoids. *Leea indica* is known to remove warts caused by the human papilloma virus.

Commented [A12]: There is no statistical analysis was done in this study?

Commented [A13]: The results and discussion is only superficial. Thorough results and discussion need to be included.

Commented [A11]: Rephrase.

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The use of plant parts in this study in several diseases may be caused by the content of phytochemical compounds present in these plant parts. Several phytochemical compounds known to have medicinal effects include flavonoids, tannins, alkaloids, and saponins. The results of flavonoid, tannin, alkaloid, and saponin content in the parts of some of these plants are presented in the figure 1, 2, and 3, respectively.

Result from figure 1 shows that *Caesalpinia sp* have the highest flavonoid level and follow by *Leea indica*, *Aglaia sp*, *Donax cenniformis*, *Tetrastigma sp*, and *Passiflora foetida*. For tannin level (figure 2), *Aglaia sp* shows the highest level and follow by *Donax cenniformis*, *Caesalpinia sp*, *Leea indica*, *Tetrastigma sp*, and *Passiflora foetida*. From figure 3, *Aglaia sp* shows the highest level of alkaloid and follow by *Passiflora foetida*, *Caesalpinia sp*, *Leea indica*, *Tetrastigma sp*, and *Donax cenniformis*. Also, figure 3 shows that *Passiflora foetida* have the highest saponin content and follow by *Leea indica*, *Aglaia sp*, *Caesalpinia sp*, *Tetrastigma sp*, and *Donax cenniformis*.

Antioxidant Activity

The result of this present study shows that all plants have antioxidant activity. There are 3 different antioxidant activity which is investigated in this study. These activities are hydrogen peroxide and hydroxyl radical scavenging activity and chelating metal of metal ions. The results present in figure 4.

Results from figure 4 shows that *Passiflora foetida* have best hydrogen peroxide scavenging activity and chelating of metal ions compare to others plants. Also, *Leea indica* shows the best activity for hydroxyl radical scavenging activity.

It is well known that oxygen in oxidative reaction is converted to radical superoxide which is catalysed by superoxide dismutase. Further reaction converted the radical superoxide to hydrogen peroxide by catalase as well as glutathione peroxidase [16]. Result from this reaction which in the presence of metal ions via Fenton and Haber-Weiss reaction is hydroxyl radical. Radical superoxide, hydrogen peroxide, and hydroxyl radical known as reactive oxygen species (ROS). These compounds are very reactive and promote a further reaction with macromolecule such as, lipid, glucose, and protein to induce the cell damage [17].

This chain reaction of ROS formation can inhibit by antioxidants. The inhibiting activity of antioxidants through several mechanisms, including scavenge the ROS or chelating the metal ions. The results indicated that *Passiflora foetida* can scavenge the hydrogen peroxide and chelating the metal ions. This activity might be correlated with the high saponin level contain in the extracts. Gulcin et al [12] research reported that saponin has better metals and superoxide ion binding than antioxidants BHA, BHT, and α-tocopherol. Meanwhile, Ashraf et al [18] stated that saponin has good antioxidant activities, although it still below *Chlorophytum borivilianum* level.

Besides *Passiflora foetida,* the medicinal plant that has antioxidant potential is Leea indica. This plant has flavonoid and saponine levels in (figure 1 and 3). Flavonoids work as antioxidant comprehensively, which covers (a) free radical interception (b) metal chelating (c) suppressing enzyme related to free radical formation and stimulating internal antioxidant enzyme [19]. Januarti et al research [20] concluded that flavonoid level positivley correlated to antioxidant activities of *Allium sativum*.

Anti-inflammatory Activity

Anti-inflammatory activity of all selected plants is determined by three different methods. First, protein denaturation method. This method describes protein damage that occurs as a result of the inflammatory process. The greater percentage means that the plant has a good anti-inflammation effect. The result from figure 5 shows that *Aglaia sp* has the most anti-inflammatory activity and follow by *Passiflora foetida*, *Caesalpinia sp*, *Leea indica*, *Donax cenniformis*, and *Tetrastigma sp*.

The second and third method that use in this study is heat and hypotonicity induced haemolysis. Haemolysis is also a marker of inflammation. The initial process of haemolysis is inflammation that causes cell injury. Inflammation can occur through several mechanisms, including oxidative stress and the release of inflammatory mediators. According to the results, the presence of plant extracts can inhibit the haemolysis which is means inhibited the inflammation. This activity may be caused by the phytochemical constituents of the plant

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extracts. These phytochemical compounds can inhibit the inflammation also through oxidative or inflammation mechanism. It is well known that those phytochemical compounds have several antioxidant activities [21]. Also, those phytochemical compounds can inhibit the formation of cytokine pro-inflammation, kinase and phosphodiesterase enzyme which play role in inflammations [22,23].

Conclusion

These findings shows that the all-plants extracts possess antioxidant and anti-inflammation activity. The antioxidant and anti-inflammation activity of the plant's extracts may be caused by some phytochemical constituents in the extracts. The phytochemical compounds which found in plants extracts are flavonoid, tannin, alkaloid, and saponin. All those compounds are responsible for hydrogen peroxide and hydroxyl radical scavenging activity, chelating of metal, and anti-

References

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Commented [A18]: What is the impact of knowing these 6 plants have these phytochemicals?

Commented [A19]: Anti-??

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Table 1. Scientific name, local names, part of plant, and medicinal activities of some selected plants from Mandiangin, South Kalimantan, Indonesia

Scientific Name	Local Names	Medicinal Activities	Used Parts
Passiflora foetida	Bilaran Kusan Kelambut Markisa Hutan	Hypoglycaemic effect	Stem
Caesalpinia sp	Sembilikan Asam Daun	Antitussive	Stem
Donax cenniformis	Bamban Batu	Antitussive	Stem
Aglaia sp	Kilayu	Antiviral for varicella and herpes zoster	Leaf
Tetrastigma sp	Ulur-ulur	Gastrointestinal bleeding and hemorrhoids	Stem
Leea indica	Mali-mali	Antiviral for Human Papilloma Virus	Fruit

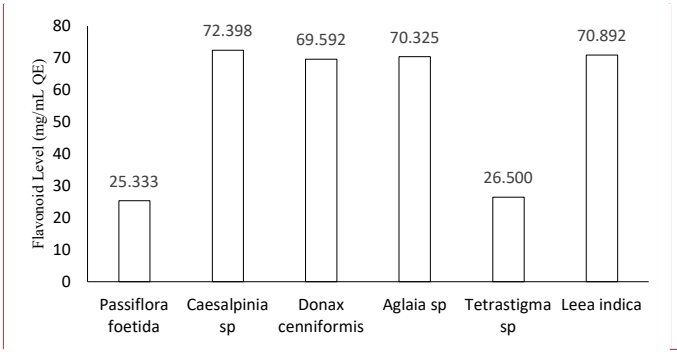

Figure 1. Flavonoid level of some selected plants from Mandiangin, South Kalimantan, Indonesia

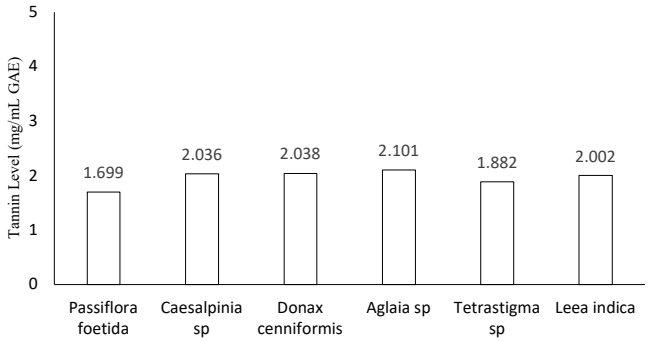

Figure 2. Tannin level of some selected plants from Mandiangin, South Kalimantan, Indonesia

Commented [A20]: Is this a mean value? Was it done one time or triplicate? It should be expressed as mean +/- SD as well as indication of statistical significance.

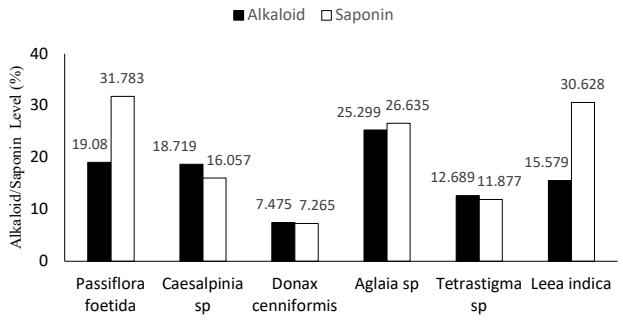

Figure 3. Alkaloid and saponin level of some selected plants from Mandiangin, South Kalimantan, Indonesia

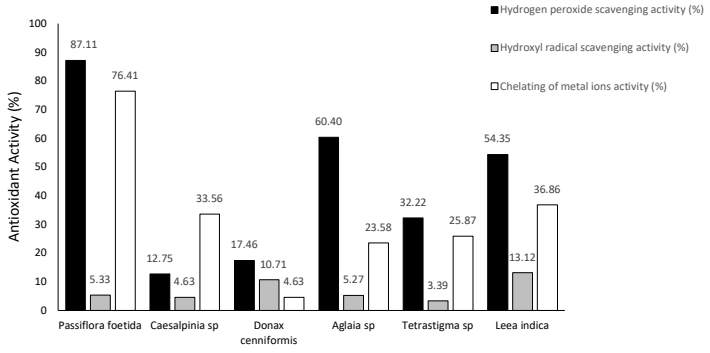

Figure 4. Hydrogen and hydroxyl radical scavenging activity and chelating of metal ions activity of some selected plants from Mandiangin, South Kalimantan, Indonesia

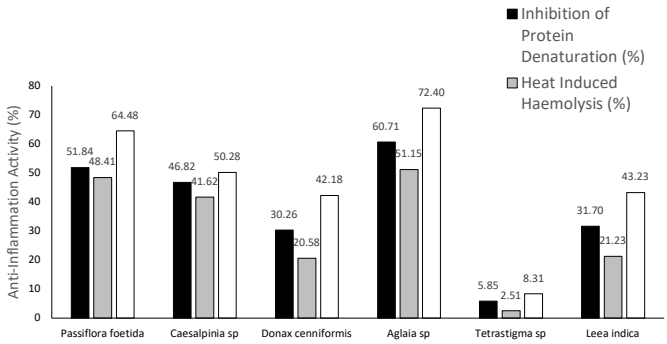

Figure 5. Anti-inflammation activity of some selected plants from Mandiangin, South Kalimantan, Indonesia