Antioxidant (gallic acid and quercetin) profile of Sumatran wild mangoes (*Mangifera* spp.): a potential source for antidegenerative medicine [version 1; peer review: 1 approved]

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Abstract

**Background:** New findings on the potential of wild mangoes from the island of Sumatra as a source of antioxidant helps their conservation effort as it introduces their useful compounds to the public. This study aims to analyze the antioxidant profile and quantification of gallic acid and quercetin content from leaves and bark of Sumatran wild mangoes. Exploration and analysis of phytochemical constituents from 11 Sumatran wild mangoes was performed.

**Methods:** Antioxidant activity of wild mangoes was analysed with 1,1-diphenyl-2-picrylhydrazyl (DPPH), and determination of quercetin and gallic acid content was performed by high performance liquid chromatography (HPLC) method. Total flavonoid and phenolic analysis was also performed. Curve fitting analysis used a linear regression approach.

**Results:** The highest level of antioxidant activity, phenolic compound and flavonoid compound was found in the leaves and bark of *Mangifera* sp1. (MBS), the bark of *M. foetida* var. batu and leaves of *M. torquenda*, and the bark and leaves of *M. sumatrana*, respectively. The content of gallic acid in leaves ranged from 5.2270-35.4763 mg/g dry weight. Quercetin content of wild mangoes leaves ranged from 0.76 to 1.47 mg/g dry weight with the lowest value in *M. foetida* var. manis and the highest in *M. laurina*.

**Conclusion:** The results obtained are expected to be useful in supporting the development of antidegenerative drugs from natural ingredients that have potential as immunomodulatory agents.

**Keywords**

antioxidant, gallic acid, quercetin, Sumatran, wild mango
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Introduction

Sumatran wild mangoes have the potential to be used as an herbal medicine and are found in the Sumatra rainforests. However, they are threatened with extinction due to rapid and massive deforestation, and due to this species lower economic market value and low consumption by humans, there is little interest in their cultivation. This could lead to an increase in the number of endangered species in the Sumatran rainforest. In nature, these species are ecologically valuable as the primary food for primates and other wildlife.

There are eight wild mango species found in Sumatra: Mangifera quadrifida, M. torquenda, M. magnifica, M. griffithii, M. kemanga, M. sumatrana, Mangifera sp1 (MBS) and Mangifera sp2. (MH)3. The conservation of wild mango types will not be effective if the benefits of these plants are unknown, while their unique characteristics show that these plants have the potential to be therapeutic agents with high antioxidant ability. Consequently, studying their ecological role and finding their potential biomedicine compounds is the best way to aid wild mango conservation.

These wild mangoes are acidic and have a strong turpentine aroma, rough fibers and strong odor, showing that these mangoes are an antioxidant source as reported by Fitiwawati et al. Antioxidants are vital to resolve some types of degenerative diseases, improving immunity and responding to external attacks, such as bacteria, viruses, fungi, and various disease-causing germs. They may even be used to manage chronic diseases, such as cancer. In the study by Taing et al., skin and flesh extracts of mangos (M. indica) had the remarkable action of inhibiting proliferation of breast and colon cancer cells.

Previous studies show that mango skin (M. indica) possess flavonol O- and xanthone C-glycosides, gallotannins and benzophenone derivatives. Mango seeds contained bioactive components such as phenolics, carotenoids and ascorbic acid, carbohydrates (58–80%), proteins (6–13%), essential amino acids and lipids (6–16%)10. The polyphenol component of mango extract is phenolic acid (gallic acid) and flavonoids (quercetin)11. These compounds are found in the edible fruits and have been shown to have great potential for protecting the body from oxidative stress-associated damages12,13.

Gallic acid (3,4,5-trihydroxybenzoyl acid) is a major polyphenolic compound present in mangoes14. Phenolic compounds are vital in the immune system response to chronic degenerative diseases, such as anti-aging, anti-inflammatory, antidiabetic and antiproliferative activities, and have a cardioprotective effect, including reduction of the side effects of chemotherapeutics15. Quercetin (3,3’,4’,5,7-pentahydroxyflavone) is one of the most abundant flavonoid-derived food compounds and is present in various mangos (e.g. M. indica)16. Flavonoid structure contains a double bond in the C ring and a 4-oxo group, which enhances its potent antioxidant activity. Flavonoids have been scientifically proven to be anti-allergic, anti-inflammatory, anti-cancer, antimicrobial, and antiviral16. Quercetin generally exists in edible plants and is mostly used in the production of traditional medicine to relieve some type of diseases17,18.

Previous research related to the antioxidant potential of wild mango species from Sumatra had been carried out qualitatively by Fitiwawati et al. Yet, this research topic needs the support of quantitative data and detailed metabolite content in order to clarify the antioxidant compound of wild mangos that have high potential to be a source of antioxidative drugs. To the best of our knowledge, quantitative analysis of the antioxidants in wild mangos has not yet been reported. Therefore, this study aimed to analyze the antioxidant compound (gallic acid and quercetin) of wild mangos from Sumatra, Indonesia in order to preserve its existence in nature. The results obtained are expected to be advantageous in the effort of discovering types that contain highly phenolic and flavonoid compounds, which will support antioxidative drug development for public health. The finding of medicinal compounds in wild mangos may make stakeholders pay attention to cultivation and restoration, so their population in nature will be conserved.

Methods

Equipment and materials

Equipment: Preparative-plate glass for thin layer chromatography, Whatman filter-paper, vacuum rotary evaporator, ultraviolet lamps 254 and 366, UV-Visible spectrophotometer (GENESYS 10S UV-VIS), High Performance Liquid Chromatography (HPLC; Shimadzu LC 20AD), cuvette, micropipette. Materials: stem bark and leaves of wild mango from Sumatra, distilled water (Batraco), ethanol (Batraco; as solvent), silica gel GF254 (Batraco; for isolation), and ethyl-acetate (Batraco; as solvent).

Plant preparation and extraction of wild mangos

The wild mango samples were collected from several provinces in Sumatra Island, such as Riau, Jambi and South Sumatera, as depicted in Figure 1. Wild mango bark was dried in an oven at 40°C, while the leaves were freeze-dried. Dried bark and leaves were ground by a blender and ~100 g of the resulting powder was macerated with 200 ml ethanol until it was submerged, and was then soaked for a day. All macerate was collected and evaporated with a rotary vacuum evaporator at 50°C to obtain a solid-liquid extract.

Quantification of antioxidant content

Antioxidant activity analysis was assessed with the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method14. Briefly, approximately 2 mg of the dried sample was dissolved in 2 mL of methanol until the concentration reached 1000 mg/mL. Then, in a plate consisting of rows A-H (each row consisted of 12 wells), 100 mL of sample was added to each well in row A. Then, a total of 50 mL methanol was added to each well for rows B to F. 50 mL was taken from row A and then added to the row B; the same volume taken from row B and added to row C; the same procedure was done to row F. 50 mL was taken from row F and discarded. The following concentrations were obtained through this process: 1000 (row A), 500 (B), 250 (C), 125 (D), 62.5 (E), and 31.25 (F) g/mL. Row G to H were filled with 50 mL of methanol, only wells 1-6 of row H were filled. Furthermore, for DPPH test, additional methanol of 80 mL (concentration of 80 mg/mL) is added to the rows A to G, and then incubated for 30 minutes. Radical binding was measured by the decrease in DPPH absorbance using a microplate reader at a wavelength of 517.
nm. A positive control was used as a comparison (ascorbic acid, 50 ug/mL).

The antioxidant activity is presented as inhibition percentage of IC\textsubscript{50} which was calculated by the formula below:\textsuperscript{25}:

\[
\%\text{Inhibition} = \left( \frac{A_0 - A_i}{A_0} \right) \times 100\%
\]

where \(A_0\) is the absorbance without sample and \(A_i\) is the absorbance of the tested sample.

Quantification of total flavonoid content
Analysis of total flavonoid content was carried out using the method of Xu and Chang\textsuperscript{26}. Quercetin solution was firstly dissolved into five concentrations (20, 40, 60, 80 and 100µg/ml) for a standard curve. Subsequently, ~100 µl of quercetin (blank) sample was mixed together with 50 NaNO\textsubscript{2}, 5% and 50 µl AlCl\textsubscript{3}.6H\textsubscript{2}O (aluminum chloride hexahydrate) 10% and 50 µl NaOH 1 M were added to a 96 well clear polystyrene microplate. The mixture was incubated in a dark place at room temperature for 30 minutes. After that, the mixture was assessed using a microplate reader at a wavelength of 510 nm. Total flavonoid content is determined by following equation:

\[
M = \frac{bD_{V}}{m_{t}} \left( \frac{m_{\text{total}}}{m_{\text{dry}}} \right)
\]

where \(b\) and \(D_{V}\) are regression coefficient and dilution factor, respectively, \(V\) is extracted volume of the sample, and \(m_{t}\) is extracted mass. Note that the mango sample is in dried form (100g used), so that \(m_{\text{total}}\) refers to total mass of extracted sample and \(m_{\text{dry}}\) indicates sample dry mass.

Quantification of total phenolic content
Analysis of total phenolic contents from dried samples of 100 gr was performed using the method of Folin-Ciocalteu from Musa \textit{et al.}\textsuperscript{27}. Gallic acid solution was initially dissolved into five concentrations (20, 40, 60, 80, and 100µg/ml) for the standard curve. About 100 µl of hydrolysis and non-hydrolysis of gallic acid as reference sample was then mixed with 50 µl of Folin-Ciocalteu reagent 0.25 in a 96 well clear polystyrene
Quantification of quercetin and gallic acid content by HPLC
Mango extract was dissolved in ethanol (100 g/100 mL). Quercetin and gallic acid standard compounds were each prepared in several concentrations of 100, 50, 25, and 12.5 µg/ml. Each standard compound was mixed with 20 µL and eluted for 20 minutes through elution gradient method (with water/methanol = 0–100 and UV detector with wavelengths of 360 and 370 nm) to obtain a standard chromatogram. The sample was then filtered using a 0.45 µm filter (13mm PTFE) and analyzed according to the HPLC method of quercetin and gallic acid. The level of quercetin and gallic acid contained in each sample was calculated from regression plot of Y= a lnX+b. This plot is described the ratio of the sample concentration to the standard chromatogram of the quercetin and gallic acid standard.

Data analysis
The content of gallic acid and quercetin in the sample is indicated by the change of the solution color in DPPH test which indicated antioxidant activity. This change was read by a microplate (Berthold, Germany) using the software of MikroWin 2000 version 4.3x. Furthermore, a linear regression curve is performed to obtained quantification of gallic acid and quercetin content from each sample.

Results
Antioxidant content
Antioxidant values in this study were determined using the DPPH method. This method is a simple, easy, fast and sensitive method. Only a small amount of natural material is used to evaluate antioxidant activity, so it is widely used to test the ability of compounds that act as electron donors. The principle of this measurement is the presence of stable free radicals, namely DPPH mixed with antioxidant compounds that have the ability to donate hydrogen so that free radicals are neutralized. Measurement of antioxidant activity using UV-vis spectrophotometry is used so that the value of free radical will be known, expressed by the value of IC<sub>50</sub> (inhibitory concentration). Table 1 contains the antioxidant activity of eight wild mango species.

Antioxidant compounds will generally react to DPPH radicals through mechanism of donation of hydrogen atoms which causes the discoloration from purple to yellow. In this study, the strongest levels of antioxidant activity in wild mangos was found in Mangifera sp1. (MBS) leaves and bark extract with IC<sub>50</sub> value of 0.88 ppm and 33.24 ppm, respectively. Antioxidant compounds at a moderate level were discovered in the extract of Mangifera foetida<sub>1</sub> (var. limus) leaves (IC<sub>50</sub> value of 196.24 ppm) and Mangifera foetida<sub>2</sub> (var. manis) bark (IC<sub>50</sub> value of 117.62 ppm).

Phenolic and flavonoid content
The phenolic and flavonoid compounds were quantified in the mangos using the method of Folin-Ciocalteu from Musa et al. and the method of Xu and Chang, respectively. Total phenolic content was represented in milligrams (mg) of gallic acid, which was equivalent to per gram of the dry weight of mango leaves (mg GAE/g) (Table 2). Total flavonoid content was represented in milligrams (mg) of quercetin, which was equivalent to per gram of the dry weight of mango leaves dry weight (mg of QE/g).

Table 1. IC<sub>50</sub> of wild mango species from Sumatran, Indonesia, using the DPPH (1,1-diphenyl-2-picryl hydrazyl) method.

| Species                  | IC<sub>50</sub> value (ppm) |
|--------------------------|-----------------------------|
| Mangifera foetida (var. limus) | Bark 60.52                  |
|                         | Leaves 196.24               |
| Mangifera foetida (var. manis) | Bark 117.62                |
|                         | Leaves 195.12               |
| Mangifera foetida (var. batu) | Bark 34.22                 |
|                         | Leaves 119.66               |
| Mangifera kemanga       | Bark 7.034                  |
|                         | Leaves 152.23               |
| Mangifera sumatrana     | Bark 10.57                  |
|                         | Leaves 8.70                 |
| Mangifera laurina       | Bark 22.51                  |
|                         | Leaves 18.25                |
| Mangifera sp1. (MBS)    | Bark 33.24                  |
|                         | Leaves 0.88                 |
| Mangifera sp2. (MH)     | Bark 45.48                  |
|                         | Leaves 2.40                 |
| Mangifera quadrifida    | Bark 28.44                  |
|                         | Leaves 26.26                |
| Mangifera torquenda     | Bark 66.87                  |
|                         | Leaves 47.62                |
| Mangifera magnifica     | Bark 41.75                  |
|                         | Leaves 6.76                 |

Gallic acid and quercetin content
Spectrum chromatogram of standard solutions and chromatograms of each species at wavelengths of 360 and 370 nm were used. Specific quantitative tests of gallic acid and quercetin on the leaves of wild mangos from Sumatra using HPLC showed that the plant leaves were positive for both bioactive compounds. The plot of standard calibration for quercetin and gallic acid is shown in Figure 2 and the results are summarized in Table 3.

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Table 2. Total phenolic and flavonoid content of wild mango species from Sumatran, Indonesia.

| Species                  | Total phenolic content (mg GAE/g) | Total flavonoid content (mg QE/g) |
|--------------------------|----------------------------------|----------------------------------|
| M. foetida, (var. limus) | Bark: 41.76                      | Leaves: 63.93                    |
|                          | Bark: 24.64                      | Leaves: 57.29                    |
| M. foetida, (var. manis) | Bark: 75.36                      | Leaves: 68.93                    |
|                          | Bark: 63.59                      | Leaves: 63.82                    |
| M. foetida, (var. batu)  | Bark: 100.65                     | Leaves: 94.34                    |
|                          | Bark: 70.83                      | Leaves: 92.50                    |
| M. kemanga               | Bark: 90.65                      | Leaves: 87.46                    |
|                          | Bark: 20.08                      | Leaves: 48.55                    |
| M. Sumatrana             | Bark: 88.43                      | Leaves: 98.69                    |
|                          | Bark: 65.72                      | Leaves: 107.50                   |
| M. laurina               | Bark: 44.10                      | Leaves: 39.00                    |
|                          | Bark: 81.07                      | Leaves: 95.36                    |
| M. sp., (MBS)            | Bark: 84.80                      | Leaves: 68.30                    |
|                          | Bark: 69.63                      | Leaves: 81.55                    |
| M. sp., (MH)             | Bark: 93.01                      | Leaves: 82.92                    |
|                          | Bark: 64.70                      | Leaves: 96.72                    |
| M. quadrifida            | Bark: 79.72                      | Leaves: 58.85                    |
|                          | Bark: 89.00                      | Leaves: 75.53                    |
| M. torquenda             | Bark: 76.30                      | Leaves: 85.10                    |
|                          | Bark: 92.48                      | Leaves: 66.65                    |
| M. magnifica             | Bark: 77.37                      | Leaves: 66.55                    |
|                          | Bark: 45.63                      | Leaves: 65.70                    |

Figure 2. Plot of standard calibration of quercetin (left) and gallic acid (right).

Discussion
Antioxidants are a group of chemical compounds that have the function of suppressing cell damage, caused by free radicals, by giving electrons speedily and transforming the free radicals into stable forms, in order to prevent oxidative damage that cause ailments. The presence of antioxidants in plants is primarily as a protective compound from pest and disease, known as bioactive compounds. In this study, the quantitative phytochemical analysis was purposely conducted to determine the level of antioxidants and content of flavonoid and phenolic compounds, specifically
Based on the results of total antioxidant values of wild mangos using DPPH assay, it was revealed that Mangifera sp1. (MBS) is the highest antioxidant activity amongst other wild mangos (bark, 0.88 ppm; leaves, 33.24 ppm). According to Badarinath et al., antioxidants are expected to be very strong if the IC50 value is smaller than 50, in the range of 50–100 ppm, yet it is weak when the IC50 value ranges between 100–250 ppm and is inactive if the IC50 is <250 ppm. The smaller the IC50 value, the higher the antioxidant activity. The antioxidants contained in mangos are essential for enhancing the immune system in the body, and are an immunomodulator agent2, including analgesic3, antimicrobial and antidiabetic properties.4,5 Fittawati et al.6 reported that powder ethanol extract of Mangifera sp1 (MBS) leaves are immunomodulatory agents with phagocytic activity at 69.67%. Ascorbic acid contained in the bark and leaves of mangos has great potential to heal chronic wounds and inflammation.6 Also, mangos are a particularly rich source of polyphenols, a diverse group of organic micronutrients found in plants, which exert specific health benefits.

The presence of gallic acid and quercetin from wild mangos in this study shows that they have potential as antioxidants of phenolic and flavonoid groups. The highest phenolic compounds of wild mangos were in the bark of M. foetida (var. batu) and leaves of M. torquenda (100.65 mg/g and 92.48 mg/g, respectively). Whereas, the lowest phenolic compounds of wild mangos were in the bark of M. foetida (var. limus) and the leaves of M. kemanga (63.9 mg/g and 48.55 mg/g, respectively). Barreto et al.7 reported that quercetin pentoside compounds in methanol extract of leaves of Van Dyke cultivated mangos from Brazil was 1.33 g/kg in old leaves and 4.93 g/kg in young leaves. In contrast, pentoside was undetectable in the bark.8 However, the present study revealed that all bark of wild mangos assessed contained quercetin. Ali et al.9 and Kim et al.10 also reported that cultivated mangos (M. indica) mostly contain flavonoids, carotenoids, vitamin E and C, terpenoids and steroids.

Table 3. Quercetin and gallic acid content of wild mango leaves from Sumatran, Indonesia.

| Species                  | Quercetin (mg/g dry weight) | Gallic acid (mg/g dry weight) | Retention time (min) Quercetin | Gallic acid |
|--------------------------|-----------------------------|-------------------------------|--------------------------------|-------------|
| M. foetida (var. limus)  | 51153                       | 144789                        | 14.97                          | 10.24       |
| M. foetida (var. manis)  | 138513                      | 198816                        | 15.64                          | 10.49       |
| M. foetida (var. batu)   | 137886                      | 123830                        | 15.71                          | 10.26       |
| M. kemanga               | 550164                      | 98147                         | 15.47                          | 10.26       |
| M. sumatrana             | 714087                      | 88518                         | 15.45                          | 10.26       |
| M. laurina               | 902079                      | 122354                        | 15.43                          | 10.23       |
| Mangifera sp1. (MBS)     | 96833                       | 115273                        | 15.56                          | 10.23       |
| Mangifera sp2. (MH)      | 227010                      | 128227                        | 15.51                          | 10.23       |
| M. quadrifida            | 230653                      | 113892                        | 15.49                          | 10.26       |
| M. torquenda             | 462008                      | 114851                        | 15.50                          | 10.28       |
| M. magnifica             | 243010                      | 111497                        | 15.48                          | 10.26       |

Based on the results of the HPLC chromatogram, the contents of the bioactive compounds of wild mangos showed that the content of gallic acid obtained from wild mangoes leaves ranged from 5.22–35.47 mg/g of dry weight, where the lowest content was in M. sumatrana and the highest was M. foetida (var. manis). According to Soong and Barlow11, mango seed extract (Mangifera indica L.) contains gallic acid (23–838 mg/100 g of dry weight, equivalent to 0.23–8.38 mg/g). Rastrallesi et al.12 also stated that gallic acid content in mango bark is ~226.2 mg/100g of dry weight, 6.0 mg/100 g in seed kernel13, and 6.9 mg/kg in mango pulp14. These results indicate that gallic acid content is higher in wild mango leaves (as seen in the present study) compared to other parts of the mango.
The study by Barreto et al.\textsuperscript{11} reported that gallic acid is the major secondary metabolite component in the flesh and seeds of mangoes (\textit{M. indica}). Gallic acid is the most abundant molecule in ‘Ataulfo’ mango peel\textsuperscript{12}. The flesh, seeds, and kernel of \textit{M. indica} are sources of gallic acid, which is a bioactive compound with potential health-promoting activity\textsuperscript{13}. Ajila and Prasada\textsuperscript{14} stated that quercetin is the major flavonoid type in the ripe peel of mango.

Gallic acid has anticancer, anti-inflammatory, antimicrobial, and antimutagenic, including radical-scavenging, activities\textsuperscript{47}. It has also been shown to inhibit colon cancer cell (HCT-15) proliferation, inhibits platelet aggregation, calcium mobilization, and tyrosine protein phosphorylation in platelets\textsuperscript{16}, and inhibits inflammatory allergic reactions\textsuperscript{48}.

Quercetin plays a role in triggering fruit color development. In the present study, \textit{M. laurina} had the highest quercetin content, while the lowest was found in \textit{M. foetida} (var. Limus). Quercetin content of wild mangoes leaves ranged from 0.76 to 1.47 mg/g of dry weight. Berardini et al.\textsuperscript{1} reported that quercetin content in mango peel was 65.3 mg/kg of dry matter, equivalent to 0.653 mg/g dry weight. Based on these data, wild mango leaves have higher quercetin content (as found in the present study) than peel. High quercetin content is found in fruits and quercetin-3-glucoside is mainly found in leaves\textsuperscript{21}.

Quercetin has various medicinal benefits, including aiding treatment in brain disorders, renal injury, cardiovascular diseases, high blood pressure, cancer, bacterial activity, inflammation, diabetes mellitus, arthritis and asthma\textsuperscript{49}. It decreases estrogen receptor cells of breast cancer, inhibits tyrosine kinase, arrests human leukemic T cell development\textsuperscript{50}, protects the liver from oxidative damages\textsuperscript{51}, prevents cardiovascular disease, and exhibits antithistamine and anti-inflammatory effects associated with various forms of arthritis\textsuperscript{52}.

Fitmawati \textit{et al.}\textsuperscript{53} demonstrated that wild mango leaves from Sumatra have immunostimulant activity and antioxidant compounds\textsuperscript{54}, including therapeutic efficacy potential for the prevention of degenerative diseases. Wild mangos from Sumatra contain flavonoids in high amounts, hence these contents need to be further explored and maximized for medicinal usage, in order to support the availability of medicinal resources. The results of the antioxidant activity using the DPPH method as assessed in the present study, and the total content of flavonoids and phenolics exhibited the varied results for each species. Wild mango species that have the highest antioxidant content may not have the highest total levels of flavonoids and phenolics because each plant species has different levels and types of metabolites.

Conclusions

This study provided quantitative information about the presence of antioxidants, as well as the specific antioxidant contents, of wild mangos. The current investigation was undertaken to quantify the percentage of phytochemicals in the leaves and bark of wild mangos as an alternative raw material for medicine. This showed that this study provides new knowledge regarding the content of gallic acid and quercetin in wild mangos, found in the leaves, which could be used as an immunomodulatory agent. The results obtained are expected to be useful in supporting the development of anti-degenerative drugs, as well as to support the conservation of wild mangos, which are rare while maintaining and improving their quality and diversity.

Data availability

\textbf{Underlying data}

Open Science Framework: Wild Mango Exploration in Sumatra for Potential Health Medicine, https://doi.org/10.17605/OSF.IO/MP6KF\textsuperscript{55}.

This project contains the following underlying data:

- Raw_Data_for_Gallic_Acid
- Raw_Data_for_Quercetin

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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The authors describe in detail a new information on antioxidant profile of 11 Sumatran wild mangoes (Mangifera spp), including flavonoid and phenolic content as well as gallic acid and quercetin of its leaves and barks. The paper is well written. A few suggestions are made, however:

Introduction: Need information why antioxidant activity is determined on leaves and barks instead of mango’s peel, pulp and seed kernel.

Method:
1. There is no information about quercetin and gallic acid standard for HPLC analysis. Need to specify the origin of quercetin and gallic acid standards.

2. No information of HPLC column, please specify the column used.

Results and discussion:
1. The last sentence of paragraph 6 stated “Ailla and Prasada stated that quercetin is the major flavonoid type in the peel of mango”, since this paragraph talk about gallic acid not quercetin, so discussion about quercetin should be moved in the paragraph 8.

2. On paragraph 8 stated the lowest quercetin was found in M foetida (var Limus), actually as we can see from Table 3, it should be M foetida_2 (var. manis) and M foetida_3 (var. batu).

3. Total phenolic is represented by gallic acid content. Increasing phenolic content should increase the gallic acid, but the study didn’t show this. Why this condition can appear? Does any others phenolic compound instead of gallic acid influence the total phenolic in mango barks and leaves?

4. Flavonoid content is represented by quercetin. Increasing flavonoid content should increase the quercetin, but the study didn’t show this. Why this condition can appear? Does any others
flavonoid compound instead of quercetin influence the flavonoid compound in mango barks and leaves?

5. IC\textsubscript{50} values indicate the antioxidant activity, the lower IC\textsubscript{50} value the higher antioxidant activity. In this study the antioxidant activity is represented by total phenolic and flavonoid content. Lowering IC\textsubscript{50} should increase total phenolic and flavonoid content, but the study didn’t show this. Please discuss this phenomena.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Active compound of agriculture product

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 13 May 2020
Fitmawati Fitmawati, Universitas Riau, Pekanbaru, Indonesia

We would to thank the reviewer for their comments to our manuscript. In this new version, we have reviewed and modified text to improve the clarity and understanding manuscript. The main changes have been:

1). Introduction the reason for using leaves and bark to determine the antioxidant value of wild mangoes is added.

2). The method has already mentioned the origin of the standard quercetin and gallic acid and the HPLC column used.

3). In the results and discussion of data changes due to input errors in the previous article. It has been discussed that there are other compounds of flavonoid and phenolic derivatives in leaves and
bark of wild mango that refer to the literature, thus affecting the amount of gallic acid and quercetin, as well as antioxidant values.

**Competing Interests:** No competing interests were disclosed.