Antioxidant Activity of Wild Mango (*Mangifera*) From Sumatra

Fitmawati*, Erwina Juliantari, Rodesia Mustika Roza, Mayta Novaliza Isda

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau
KampusBinawidya Jl. HR. Soebrantas Km 12.5 Pekanbaru 28293, Riau, Indonesia
fitmawati2008@yahoo.com

*Corresponding author

Abstract: Wild mango is a species of mango that is agronomically and economically less valuable due to the characteristics of sour taste, thick fibres and thin mesocarp, that are less attractive to be consumed in comparison with common mango. These characteristics with low consumptive values make this fruit difficult to be cultivated. This study aims to determine the antioxidant content of wild mango. The qualitative analysis of antioxidant activity in wild mango used both method of TLC (Thin Layer Chromatography) and HPLC (High Performance Liquid Chromatography), whereas the quantitative analysis used DPPH (1,1- diphenyl-2-picryl hydrazyl) Method. The qualitatively antioxidant activity of wild Mango from Sumatra using TLC and HPLC methods showed the presence of bioactive contents. The quantitively antioxidant activity with IC_{50} resulted that *Bukit suligi'*s mango had the highest level of antioxidant activity and *Mangifera torquenda* had the lowest level of antioxidant activity both in the stem or leaves. The antioxidant compounds found in wild mango could improve the phytochemical insight and be potential for the phytopharmaceuticals of antidegenerative treatments.

Keywords: Antioxidant, Mango (*Mangifera*), Sumatra, Wild.

1. Introduction

Wild mangoes are the species that has no economic value as they have poor characteristics as an appetising fruit that makes them not suitable for being consumed daily. Thus, it becomes a challenge for its cultivation. However, wild mangoes are believed to have a high acidity level of its characteristics that would be potential as an antioxidant for a therapeutic agent in comparison for the common mangoes.

The spesies of wild mango from Sumatera consisted of *Mangifera quadrifida*, *M. torquenda*, *M. magnifica*, *M. grifithii*, *M. kemanga*, *M. sumatrana*, *Mangifera* sp_1 (*Bukit suligi* mango) and *Mangifera* sp_2 (*hutan* mango) [8]. According to Mohan et al. (2013), the mango species of*Mangifera indica* contains of some phytochemical compounds such as saponins, terpenoids and anthraquinone and the content of active therapeutic compounds, namely mangiferin, stigmasterol, friedelin and lupeol [13]. Ribeiro et al. (2010) reported that *M. indica* is a good source of antioxidants content for humans such as carotenoids, ascorbic acid and beta-carotene [22]. The total of polyphenol and flavonoid contents showed big variations in mango's genotype and are strongly correlated with the total antioxidant capacity. It was concluded that the significant genotypic differences were presented in the total antioxidant capacity of mangoes. Both polyphenols and flavonoids levels are the main contributory compounds for the total antioxidant capacity of mangoes [26].

Free radicals are considered to play a role in the process of disease in the human body. The imbalance content between free radicals and antioxidant levels in the body becomes the cause of the free radicals being dominant in the body, resulting in various diseases such as cancer, diabetes, liver, aging [10], heart issues, metabolic disorders [4] and neurodegenerative [20]. Antioxidants are the oxidation reaction – preventable compounds either in the body or with other chemical compounds that are easily oxidised [7]. According to Wu et al. (2017), antioxidants are able to donate one or more its electrons to the free radicals, resulting the risk reduction of the free radicals to be less harmful [25]. At present, the exposure of free radicals is relatively extensive in people due to air-pollution problems and diet issues [6].

The several cultivated species of mango in Indonesia as a potential source of antioxidants have been known well, namely species of *Mangifera indica* L. var. gedong [21]. Therefore, it is important to study the antioxidant potency of wild mangoes, especially from Sumatra in the context of preserving their existence. The results of research obtained on the benefits of wild mangoes for anti-degenerative

treatment will provide new information to the large community so that it can support efforts to conserve wild mangoes whose status is already scarce while maintaining and improving the quality and diversity of their values.

2. Material and Methods

2.1. Materials

The tools used in this study were a preparative-plate glass of thin layer chromatography, Erlenmeyer 250 mL, measuring glass 10 mL and 100 mL, beaker glass, electrical balance, a stirrer, capillary tube, saturated paper, *Whatman* filter-paper, test-tubes, volume-pipette, measuring pipette, filter, blender, separating funnel, flask, vacuum rotary evaporator, ultraviolet lamps 254 and 366, UV-Visible spectrophotometer (GENESYS 10S UV-VIS), HPLC (Shimadzu LC 20AD), cuvette, micropipette, yellow and blue tip, and a pipette.

Materials used in this study were the samples of wild Mango from Sumatra, mangos rinds (*Garcinia mangostana*), vitamin C, Drug Stimuno, distilled water, methanol, silica gel GF254, ethyl-acetate, DPPH powder (1,1-Diphenyl-2-picrylhydrazyl).

2.2. Sample Extraction

2.2.1. Wild Mango extraction with Aquades solvent
Each samples of wild Mango, at weight of 250 g, was crushed to powder by using a grinder. This powder was then used for the extraction of secondary metabolite constituents by adding 1 litre of distilled water into a jar containing 250 g of wild mango powder until the powder was submerged thoroughly. After that, it was then soaked for 1 hour. The extraction was filtered with the filter paper. The filtrate was then evaporated to form a solid-liquid extract which will be used for the future test of antioxidant activity.

2.2.2. Wild Mango extraction with methanol solvent
The simplicia of wild Mango from Sumatra was grinded by using a blender. Then, the powder was obtained and could be used for the extraction. An estimated of 100 g of the simplicia powder was macerated with methanol solvent until it was submerged, and being soaked for a day. All macerate was collected and evaporated with a rotary vacuum evaporator at 50 °C to obtain a thick extraction. The resulted extract was then measured for the volume obtained.

2.3. The Antioxidant Activity in Quantitative and Qualitative of Wild Mango Extraction

2.3.1. The qualitatively antioxidant activity test of wild Mango with Thin Layer Chromatography Methods (TLC)
An estimated of 0.5 ml liquid extraction (from point A) was then inserted into the vial. The total of 5 samples were prepared for the TLC plate with a length of 5 cm elution. The respective samples were applied to the initiator layer in the elution from the chamber to the termination lines. The pattern of spots appeared on the lamp resulted from UV's ray at separation λ = 254 nm and 336 nm.

2.3.2. The qualitatively antioxidant activity test of wild Mango with High Performance Liquid Chromatography (HPLC)
The HPLC analysis was performed using the elution method. The samples were dissolved in methanol (HPLC grade) (1 mg in 1mL methanol) and then filtered with an estimated of 0.45 µ of 13mmPTFE. The volume of 20 mL filtrates were injected into the column, and then the samples were analysed using several solvents ratio of water: acetonitrile for 25 minutes (HPLC grade at 1 mg in 1mL methanol). ODS column was detailed with a length and a diameter of 150 x 4.6 mm.

2.3.3. The quantitatively antioxidant activity test of wild Mango with DPPH (1,1- diphenyl-2-picryl hydroxyl) Method
The test of antioxidant activity used a microplate reader with two-fold-dilution on the DPPH method (1,1- diphenyl-2-picryl hydroxyl) (Zhang et al., 2006) at 520 nm. A sample of 2 mg was dissolved in 2 mL of MeOH until the concentration of the sample became 1000 mg/mL. The line A was inserted a

sample of 100 mL (the plate consisting of rows A-H, respectively with 12 wells of each rows). A total of 50 mL MeOH was inserted into each wells from the row B to F. The volume of 50 mL taken from the row A was then added into the row B; the same volume of 50 mL taken from the row B was subsequently inserted into the row C; the same procedure was continually done to the row F. However, the total volume of 50 mL taken from row F was discarded in order to obtain a concentration of 1000, 500, 250, 125, 62.5, and 31.25 g/mL. In the other hand, the row G to H were filled with 50 mL of MeOH, especially only wells on the line 1 to 6 of row H filled. The row A to G for DPPH method was added by 80 mL of MeOH with a concentration of 80 mg/mL, and then incubated for 30 minutes. The activity of radical bounding was measured as a decrease of DPPH absorbance through the presence of microplate reader and data processing. Positive control was used as a comparison for ascorbic acid gradient at a concentration of 50 ug/mL. The percentage of inhibition value was calculated by the following formula:

$$\% \text{ Inhibition} = (\text{A Control-A Sample})/(\text{A Control}) \times 100\ \% \quad (1)$$

Where:

A control = Absorbance uncontained sample
A sample = Absorbance sample.

3. Results and Discussions

3.1. Results

3.1.1. TLC Methods *(Thin Layer Chromatogram)*

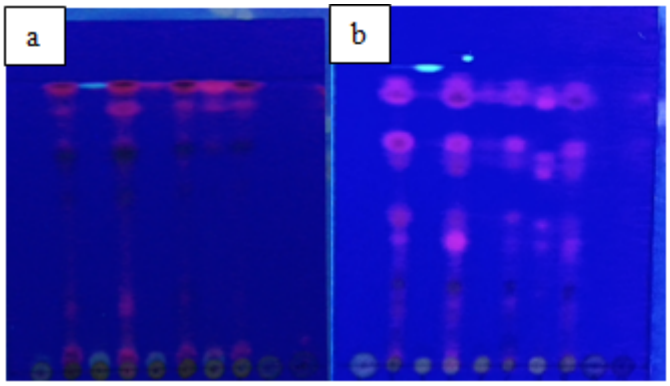
The qualitative test of antioxidant activity by TLC method with suitable mobile phase using DPPH 0.2% created spotting in methanol. Antioxidant elements found in a positive context for DPPH reagents with regards of yellow spots with a purple background. The results of extraction qualitative test of the antioxidant activity with DPPH method are shown in the Figure 1.

Extraction of samples were seen on TLC plates showing the different patterns from UV lamps and wavelength conditions. The extraction using n-hexane: ethyl acetate (8:2) eluent was measured at λ 254 nm of UV wavelength and the extraction using n-hexane: ethyl acetate (7:3) eluent was measured at λ 366 nm of UV wavelength. The use of several eluents was expected to obtain the flavonoid contents that exist in sample extraction [2]. The reason for using this n-hexane and ethyl acetate eluent was, this procedure can be done clearly and by various based on the TLC plate; the properties of this eluent are likely non-polar. The concept of separation and non-polar compounds follows the TLC principle, "likes to dissolve like" [6].

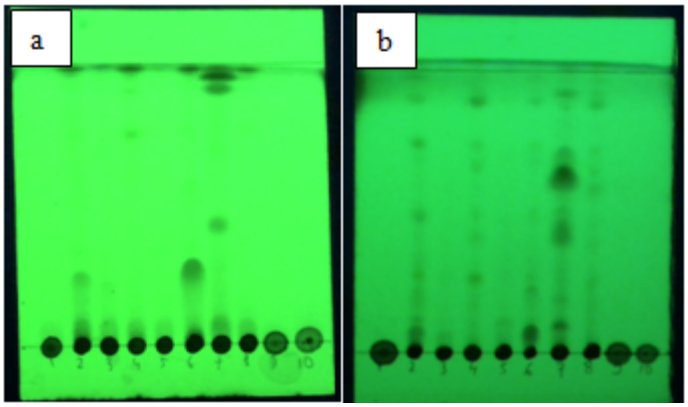
Based on the TLC results, it can be compared between spots of wild mango samples with different wavelengths, i.e. UV λ 254 nm and UV λ 366 nm. These results indicate that more than one point indicates that the sample has organic compounds or antioxidant compounds. At a wavelength of 254 nm the antioxidant compound is not clearly visible. However, at a wavelength of 366 nm, an antioxidant compound can be seen. One of them is the KLT plate using hexane: ethyl (6:4) eluent, that is the spot is more clearly visible than other eluents. Based on the figure, it can be seen that the components of n-hexane, ethyl acetate and ethanol extract play a role in antioxidants in reducing their free radicals [21].

3.1.2. HPLC Methods (*High Performance Liquid Cromatography)*

Based on the chromatogram results from HPLC with a wavelength of 254 nm and 366 nm. The wavelength is a suitable measure for identifying flavonoids and phenolic compounds. The wild species of mango samples showed the presence of secondary metabolites which can be seen from the single peak shown in the chromatogram. Based on the results of the HPLC chromatogram, the intensity of different bioactive compounds is produced. *M. torquenda* is one of the wild mangoes that have the highest levels of bioactive compounds among other wild mangoes.



(a) Eluentn-hexane : Ethyl = 8 : 2 (b) Eluentn-hexane : Ethyl = 6 : 4
Wavelength 366 nm



(a) Eluentn-hexane : Ethyl = 8 : 2 (b) Eluentn-hexane : Ethyl = 6 : 4
Wavelength 254 nm

Figure 1. TLC profile of mango which have given DPPH with Eluent ethyl-acetate= 100 % and Eluent ethyl-acetate: Methanol = 6:4. Note: 1. *Mangifera torquenda* Bark; 2. leaf; 3. *Bukit suligi* Mango Bark; 4. leaf; 5. *M. quadrifida* Bark; 6. leaf; 7. *Hutan* Mango Bark; 8. leaf; 9. *M. magnifica* Bark; 10. leaf.

Table 1. The intensity of antioxidant compounds in wild mango

Sample		Wavelength	
		254 nm	366 nm
Mangifera torquenda	**Stem**	**11,394**	**11,393**
	Leaf	10,841	10,831
Bukit Suligi	**Stem**	**3,821**	**3,818**
	Leaf	3,808	3,809
Mangifera quandrifida	Stem	3,204	3,802
	Leaf	**9,857**	**10,434**
Mangga Hutan	**Stem**	**10,896**	**10,897**
	Leaf	3,836	3,829
Mangifera magnifica	Stem	3,840	3,840
	Leaf	**3,936**	**3,921**

3.1.3. Metode DPPH *(1,1- diphenyl-2-picryl hydrazyl)*
Quantitative test in measuring antioxidant activity using DPPH method. The amount of antioxidant activity is indicated by IC_{50} values, namely the concentration of sample solution needed to inhibit 50% DPPH free radicals. The results showed that there was a relationship between free antiradical activities

on DPPH if the lower IC_{50} value, the inhibitory power of the extract against free radicals was higher. Classification of antioxidant activity based on the IC_{50} value obtained, that is very strong (IC_{50}<50 ppm), strong (50 ppm<IC_{50}>100 ppm), Medium (100 ppm<IC_{50}>150 ppm), weak (150 ppm<IC_{50}>200 ppm) and very weak (IC_{50}>200 ppm) [15].

Table 2. LC_{50} Value of wild mango

Mango	IC_{50}(ppm)	
	Stem	Leaf
Mangifera torquenda	66.8700	47.6272
Mangiera quandrifida	28.4446	26.2673
Hutan Mango	45.4812	2.4053
Mangifera magnifica	41.7578	6.7644
Bukit suligi Mango	33.2422	0.8796

Based on Table 2, the highest activity level of various species of mangoes is very strong in the part of the mangoes, namely in the *bukit suligi* mango of 0.8796 ppm followed by *hutan* mango of 2.4053 ppm and *M. magnifica* of 6.7644 ppm. Whereas in the stem, the strongest antioxidant content occurred in *M. quandrifida* at 28.4446 ppm followed by *bukit suligi* mango at 33.2422 ppm. The lowest level of antioxidant activity on the bark and leaf is *M. torquenda* with the value 66.8700 ppm and 47.6272. Therefore, it can be concluded that the *bukit suligi* mango has the strongest level of antioxidant activity and *M. torquenda* has the best antioxidant activity level on the stems and leaves. This is in accordance with the research of Rahmiyani & Nurdianti (2016) which states that the *M. indica* L. species has a high antioxidant in n-Heksana solvent of 10.11 ppm [21].

3.2. Discussion

Free radicals are molecules with an extremely reactive odd number of electrons that are widely spread, oxidation and peroxidation of proteins, lipids and DNA which will cause cell and tissue damage [13]. Many research developments in antioxidant have examined therapeutic potential in preventing disease. Antioxidants are compounds that can prevent from the oxidation of other compounds which occurs either in the body or interaction of other compounds which are easily oxidized [6].

In this study, the bark and leaf extract of wild Mango from Sumatra was evaluated for antioxidant by DPPH method. The presence or absence of antioxidant compounds was carried out by TLC method (Thin Layer Chromatogram) and the magnitude of the antioxidant compound was known from testing using the HPLC (High Performance Liquid Cromatography) method. The DPPH method is based on a decrease in DPPH radical uptake through a hydrogen atom donation mechanism from antioxidant compounds. Antioxidant compounds neutralize the properties of DPPH free radicals by transferring either electrons or hydrogen atoms to DPPH, which inhibits them by changing the colour of DPPH solution from violet to yellow [18]. This change shows the efficiency of free radical scavengers [14].

The antioxidant activity method from five species of wild Sumatra mango obtained by all species of mango contain different antioxidant compounds. *Bukit suligi* Mango is one of the wild mangoes that have the greatest antioxidant activity among other wild mangoes on the stems and leaves. Mango is also a particularly rich source of polyphenols, a diverse group of organic micronutrients found in plants which exert specific health benefits. Base on Ali et al., (2012) and Kim et al., (2010), Mango contain flavonoids, carotenoids, vitamins E and C, terpenoids and steroids. Mangos have high antioxidant activity and effectively capture free radicals [1,11].

Antioxidants contained in mangoes are important for enhancing the body's immune system in charge of responding to or responding to attacks from outside the body such as bacteria, viruses, fungi, and various diseases that cause disease (imuno modulator) [5], analgesic [9], antimicrobial [23], and antidiabetic [3]. Ascorbic acid contained in the bark and leaves has the potential to heal chronic wounds and inflammation [17]. Mahmoud-Awny et al. (2015), reported that bioactive compounds mangiferin can reduce gastric ulcer in mice [12]. The antioxidant content can even deal with

degenerative diseases such as cancer and have a chemo preventive role that is the inhibitory effect on growth, induction of cell death and differentiation [24].

From this study, that summarised the most accurate evidence of the mango and its phytochemicals that have received a great deal of attention because of their beneficial potential in counteracting either the pro-inflammatory molecules production associated to degenerative disease, such as cancer, cardiovascular diseases, aging, and neurodegenerative disorders [10].

The reported investigations were compared to those known for wild mango from Sumatra. The antioxidant compounds found in wild mango could improve the knowledge photochemical and achieve the production of phytopharmaceuticals to associate with the most common therapies for some human disease treatments. With regard to the bio agronomic aspect, the acquisition of more information about mango's potential, as well as the development of new supply chain strategies, could be of relevance for the wild mango agricultural system.

4. Conclusion

The qualitatively antioxidant activity of wild Mango from Sumatra using TLC and HPLC methods indicated the presence of bioactive compound contents. Quantitatively antioxidant activity of IC_{50} values obtained that *Bukit suligi* mango had the strongest level of antioxidant activity and *Mangifera torquenda* had the lowest level of antioxidant activity both in the stem and leaves. These findings justify the traditional use of wild mango stem bark and leaves in traditional medical practices.

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