



## Research Article

### QUALITATIVE ANALYSIS AND BIOLOGICAL SCREENING OF *STERCULIA TRAGACANTHA* FRUIT EXTRACT

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#### ABSTRACT

Utilization of natural materials as an alternative medicine in warding off diseases is increasingly in demand. This study aimed to determine the secondary metabolite content, antioxidant activity, antibacterial activity, fatty acid content and amino acid content of the extract of *Sterculia tragacantha* fruit. Sample was evaluated for phytochemical content, antioxidant activity, antibacterial activity, fatty acid content and amino acids. The results indicated that the phytochemical compounds of papaya *woton* fruit simplicia include flavonoid, tannin, hydrolyzable tannin, phenolic, polyphenols and saponin. The extract of methanol has a strong antioxidant activity while that of n-hexane has a potential as antibacterial to *Vibrio alginolyticus*. The main fatty acids of *Sterculia tragacantha* fruit extract were methyl palmitate and methyl tricosanoate while the main amino acids were L-Arginine, L-Proline and L-Glutamic acid.

**Keywords:** Phytochemistry, Flavonoids, Antioxidants, Antibacterial, Fatty Acid, Amino Acid

#### INTRODUCTION

Utilization of medicinal plants to treat various types of diseases is increasingly favored by the community because it is relatively rare to cause unwanted side effects<sup>1</sup>. Free radical is a compound or molecule containing one or more unpaired electrons in its outer orbit. The presence of unpaired electrons causes the compound to be highly reactive looking for pairs by attacking and binding the molecular electrons around them. As the results, there occurs cell function disturbance, cell structure damage; modified molecules that cannot be recognized by the immune system and even mutations. All forms of the disorders can trigger the emergence of various diseases such as degenerative diseases to cancer<sup>2</sup>. Antioxidants are chemicals that prevent the oxidation of other chemicals. They protect key cell components by neutralizing the damaging effects of free radicals, which are a natural byproduct of cell metabolism<sup>3</sup>. Natural antioxidants come from every part of plants such as in barks, trunks, leaves, flowers, fruits and roots<sup>4</sup>. The main character of antioxidant compounds is its ability to capture free radicals<sup>5</sup>. These free radicals can oxidize nucleic acids, proteins, fats and even cell DNA as well as initiate the onset of degenerative diseases<sup>6</sup>. Use of antioxidants was known to prevent damage to body cells and food components<sup>7</sup>. According to<sup>8</sup>, the compounds that had potential as antioxidants were generally the compounds of flavonoids, phenolics and alkaloids.

One of plants that have the potential as a natural antioxidant was *Sterculia tragacantha*. *Sterculia tragacantha* plant was one of the endemic plants existing in Papua<sup>9</sup>. The results of study<sup>10</sup> suggested that the leaves of *Sterculia tragacantha* contain flavonoids, tannins, phenols and polyphenols. Secondary metabolite compounds contained in *Sterculia tragacantha* leaves was allegedly also contained in the fruit that can be efficacious as an antioxidant. This study aimed to determine the secondary metabolite content, antioxidant activity, antibacterial activity,

fatty acid content and amino acid content of the extract of *Sterculia tragacantha* fruit.

#### MATERIAL AND METHODS

##### Sample Handling

Sample of *Sterculia tragacantha* fruit was taken from Gag Island, Raja Ampat, West Papua. The sample was then cleaned and dried naturally by being aerated for 7 days. The dried samples were mashed/ ground using a machine and filtered with a mesh size of 65, and thereafter stored for further test.

##### The Phytochemical analysis

The respective anti-nutritive factors such as flavonoids, terpenoids, tannins, phenols, polyphenols, dan saponine were evaluated according to the standard chemicals<sup>11</sup>. This analysis was carried out in the UPT. Materia Medica Batu, Batu City, East Java.

##### Flavonoid Test with TLC Spectrophotodensitometry

Firstly, two 10 x 10 cm plates were washed and activated. The initial droplets (stains) were made 10 mm from the left edge and 10 mm from the bottom edge of the plates with the band width of 3 mm and the distance between the stains of 6 mm. All the stains were dropped on the two separate plates. The first plate was eluted with a TE system motion phase and the second plate was eluted with a TF system motion phase. The chamber was saturated before being eluted for 30 minutes. The elution was carried out to a distance of 8 cm and the plates were then dried at 60° C for 10 minutes in the oven. The dried plates were scanned with a TLC-Scanner 3 spectrophotodensitometer (Camag-Mutensz-Switzerland) at 210 nm wavelength and the spectra of each peak

were read in the range of 190 – 400 nm wavelengths and tested for its spectrum purity. Rf: 0.85-0.90 (Quercetin); Rf: 0.60-0.65 (Quercitrin); Rf: 0.45-0.50 (Hyperoside); Rf: 0.25-0.30 (Rutin).

### Extraction

In this research, there were two different types of solvents, namely methanol (polar) and n-hexane (non-polar). The use of the two types of solvents was intended to extract both polar and nonpolar chemical components and to know the antioxidant properties of *Sterculia tragacantha* fruit in each solvent. Extraction method used in this research was maceration extraction. The extraction was done with a ratio between the sample and solvent of 1:3 for 48 hours at room temperature<sup>12</sup>. The extract was then filtered with filter paper using a vacuum filter tool. The filtrate was hereafter evaporated at 40° C to obtain the solid extract and further stored at 0° C for further tests.

### Determination of Antioxidant Activity with DPPH Method

In this section, the first step was making a 0.2 mM DPPH solution in pro analyst ethanol. The next step was making a sample stock solution with 1000 ppm concentration of pro analyst ethanol, which was then diluted to obtain the sample solution with a series of concentration of 5, 10, 15, 20, and 25 ppm for extraction results using methanol solvent, the sample solution with a series of concentration of 50, 100, 150, 200 and 250 ppm for extraction results using n-hexane solvent and solution with a series of concentration of 2, 3, 4, 5, and 6 ppm for pure vitamin C. After that, 4 ml sample solution was taken at each concentration, which was then reacted with 1 ml of the 0.2 mM DPPH solution. The subsequent step was measuring its absorbance at 517 nm wavelength. It was thereafter made a blank solution containing no sample (4 ml pro analyst ethanol with 1 ml DPPH).<sup>13</sup> After that, the damping percentage (% inhibition) was calculated with the following formula:

$$\text{Inhibition(\%)} = \frac{\text{Abs. Blank} - \text{Abs. Sample}}{\text{Abs. Blank}} \times 100 \%$$

Regression between % inhibition and concentration of the solution obtained this following equation:

$$Y = a(x) + b$$

#### Description:

- Y states the sought value of IC (inhibitor concentration), which is 50; and
- X states the value of IC<sub>50</sub>. The value of IC<sub>50</sub> states the concentration of sample solution needed to reduce DPPH by 50%.

### Antibacterial Activity Testing

In this research, the bacteria used were those infecting fishes such as *Aeromonas hydrophila* bacteria that can cause ulcer disease in fresh water fishes, *Vibrio harveyi* bacteria that can cause diseases in shrimps, *Vibrio alginolyticus* bacteria that can cause ulcer disease in sea fishes, especially grouper, and *Vibrio parahaemolyticus* that can cause diseases in shrimps such as white feces diseases and EMS.

The antibacterial activities testing of papaya *woton* fruit extract using Kirby-Bauer method (Disk Diffusion Method) with a whole or well technique. 1 ml bacterial suspension of *Aeromonas hydrophila*, *Vibrio harveyi*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus* was each inserted into a sterile petri dish, which was here after mixed with 20 ml CMC-agar media, homogenized and then allowed to solidify. The media that had

been dense were hollowed with a diameter of 7 mm and then filled with the extract of *woton* papaya fruit with various levels of concentration aseptically. The filled media of the test preparation were subsequently incubated at 37° C for 2 x 24 hours to be then observed and measured its inhibitory zone.

### Testing of Fatty Acid Fruit Profile of *Sterculia tragacantha* fruit with LCMS

#### Sample Preparation

In the first step sample was weighed ± 2.5 gram. After weighted, the sample was inserted into a 50 mL threaded-reaction tube and added with HCl 6 N of 20 mL. The next step was hydrolyzing it in an autoclave with a temperature of 110° C for 12 hours, neutralizing it with NaOH 6N and adding it up to 50 mL. Thereafter, 0.22 µM filtration was made and the filtrate was then 10 times diluted and its 2 µL was further was injected in LCMS

#### Mobile Phase

A: 0, 1% Pentadecafluorooctanoic Acid (PDFOA) 99, 5%: 0, 5% Water/CH<sub>3</sub>CN with 0, 1% Formic acid  
 B: 0, 1% PDFOA, 10%: 90% Water/CH<sub>3</sub>CN with 0, 1% Formic acid  
 Flow: 0, 6 mL/min

### Testing Amino Acid Profile of *Sterculia tragacantha* Fruit with GC-MS

GC-MS characteristics used were Shimadzu with QP2010S type, 280 °C injector temperature, split mode injector, 1 minute sampling time, 40-270°C column temperature with the initial temperature setting of 40°C hold on for 5 minutes, which then took 10 minutes for reaching the highest temperature of 270 °C (23 °C/ minute) hold on for 60 minutes so that it was needed a total time of 88 minutes to reach 270 °C temperature, detector temperature of 280 °C, interval temperature of 250 °C, He-carrier gas, main pressure of 500-900, Flow control mode pressure, pressure of 10.9 Kpa, total flow of 58.8 ml/ m, column flow of 0.55 ml/m, linear acceleration of 26.0 cm/ s, cleaning flow of 3.0 ml/ m, split ratio of 99.8, Rtx-5MS column type, length column of 30.00 m, thickness of 0.25 µm, diameter of 0.25, and EI (Electron Impact)-ionizer type of 70 eV.

## RESULTS AND DISCUSSION

### Phytochemical Content of *Sterculia tragacantha* fruit

The test results of papaya *woton* fruit simplicia (*Sterculia tragacantha*) showed that it contains phytochemical compounds, namely flavonoid, tannin, hydrolyzable tannin, phenolic, poly phenol and saponin (Table 1).

### Results of Flavonoid Test with TLC Spectrophotometry

Based on the results of TLC presented in Figure 1, *Sterculia tragacantha* fruit contains Quercetin and Routine types of flavonoids.

### Antioxidant Activity of *Sterculia tragacantha* fruit Extract

The methanol extract of the papaya *woton* fruit had a strong antioxidant activity with IC<sub>50</sub> 1.89 ppm while the n-hexane extract had a weak antioxidant activity with IC<sub>50</sub> 294.54 ppm compared with pure vitamin C IC<sub>50</sub> of 3.55 ppm (Table 2). Antioxidants play an important role in food preservation by

inhibiting the oxidation process and contributing to the health promotion provided by many dietary supplements, nutraceuticals and functional food<sup>14</sup>.

#### Antibacterial Activity of *Sterculia tragacantha* fruit Extract

Based on the antibacterial activity of *Sterculia tragacantha* fruit of 4 types of bacteria tested, only the n-hexane extract shown antibacterial potency against *Vibrio alginolyticus* with intermediate inhibitory responses (Table 3).

Table 1: Phytochemical Compound Test Results of *Sterculia tragacantha* Fruit

No.	Phytochemical Test	<i>Sterculia tragacantha</i> Fruit	Standard
1	Flavonoid	+	Darker red or pink was developed
2	Terpenoid	-	Bluish green was developed
3	Steroid	-	Orange or brownish orange was developed
4	Mayer's reagent	-	White sediment was developed
5	Alkaloid Dragendorff	-	Orange sediment was developed
6	Tannin	+	Blackish brown, blackish blue, or blackish green was developed
7	Hydrolyzable Tannin	+	Blackish brown, blackish blue, or blackish green was developed
8	Catechol Tannin	-	Red precipitate was developed
9	Phenolic	+	Blackish brown, blackish blue, or blackish green was developed
10	Polyphenol	+	Blackish brown, blackish blue, or blackish green was developed
11	Saponin	+	Permanent (not lost) foam was developed

-, absent; +, present

Table 2: IC<sub>50</sub> Antioxidant Activity of *Sterculia tragacantha* Fruit

Extraction Solvent	IC <sub>50</sub> (ppm) ± SD
Methanol	1.89 ± 0.21
n-Hexane	294.54 ± 3.66
Pure Vitamin C	3.55 ± 0.04

Table 3: Results of Antibacterial Test of *Sterculia tragacantha* Fruit

Solvent	Type of Bacteria	Material Concentration (%)	Inhibitory Zone (mm)	Inhibitory Response
Methanol	<i>Vibrio alginolyticus</i>	12.5	0	Resistant
		25	0	Resistant
		100	7	Resistant
		K (+)	12	Sensitive
	<i>Vibrio harveyi</i>	12.5	0	Resistant
		25	0	Resistant
		100	11	Intermediate
		K (+)	18	Sensitive
	<i>Vibrio parahaemolyticus</i>	12.5	0	Resistant
		25	0	Resistant
		100	9.5	Intermediate
		K (+)	14.5	Sensitive
	<i>Aeromonas hydrophila</i>	12.5	0	Resistant
		25	0	Resistant
		100	11	Intermediate
		K (+)	24	Sensitive
n-heksan	<i>Vibrio alginolyticus</i>	12.5	30*	Intermediate
		25	30*	Intermediate
		100	24*	Intermediate
		K (+)	11.5	Sensitive
	<i>Vibrio harveyi</i>	12.5	0	Resistant
		25	0	Resistant
		100	0	Resistant
		K (+)	19	Sensitive
	<i>Vibrio parahaemolyticus</i>	12.5	0	Resistant
		25	0	Resistant
		100	0	Resistant
		K (+)	14.5	Sensitive
	<i>Aeromonas hydrophila</i>	12.5	0	Resistant
		25	0	Resistant
		100	0	Resistant
		K (+)	22	Sensitive

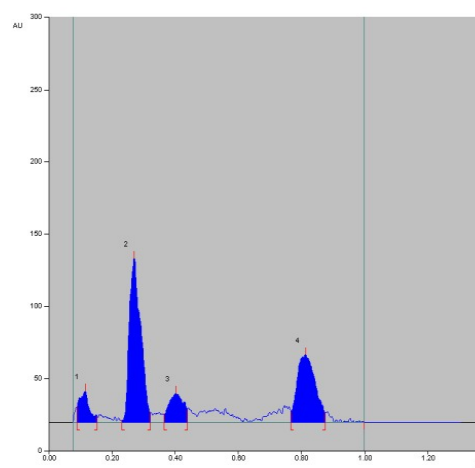
\*) The inhibitory is rather biased

Table 4: Fatty Acid Components of *Sterculia tragacantha* Fruit Extract

Retention Time (min)	Compound Name	Molecular Formula	Relative Area (%)	Amount (µg/ml)
8.299	Methyl decanoate	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>	0.83	55.5536
13.897	Methyl laurate	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	2.45	171.1342
19.233	Methyl tetradecanoate	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	3.52	273.4680
20.226	Myristoleic acid methyl esters	C <sub>15</sub> H <sub>28</sub> O <sub>2</sub>	0.88	77.9182
21.719	Methyl pentadecanoate	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	1.92	163.6075
23.607	Methyl palmitoleate	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	0.89	82.9733
24.11	Methyl palmitate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	42.00	3471.6968
26.535	Methyl heptadecanoate	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	1.30	155.0387
29.654	Methyl octadecanoate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	5.59	514.7745
30.304	Trans-9-elaidic acid methyl esters	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	7.14	833.2421
32.144	Linolelaidic acid methyl esters	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	6.99	956.5023
43.078	cis-11,14,17-Eicosatrienoic acid methyl esters	C <sub>21</sub> H <sub>36</sub> O <sub>2</sub>	1.06	189.8459
43.258	Methyl cis-5,8,11,14,17-Eicosapentanoate	C <sub>21</sub> H <sub>32</sub> O <sub>2</sub>	1.97	383.1137
43.466	Methyl erucate	C <sub>23</sub> H <sub>44</sub> O <sub>2</sub>	0.80	117.4563
43.745	Methyl docosanoate	C <sub>23</sub> H <sub>46</sub> O <sub>2</sub>	1.71	218.2087
46.687	Methyl tricosanoate	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>	19.11	2838.6289
47.581	Methyl lignocerate	C <sub>25</sub> H <sub>50</sub> O <sub>2</sub>	1.84	227.2651

Table 5: Fatty Acid Compounds of *Sterculia tragacantha* Fruit Extract

No.	Compounds	Acquisition (µg/gram)
1	L-Arginine	185.58
2	L-Valine	34.97
3	L-Proline	58.06
4	L-Alanine	31.23
5	L-Lycine	34.97
6	L-Aspartic acid	28.4
7	L-Glutamic acid	38.32
8	L-Threonine	16.8
9	L-Leucine	7.6
10	L-Isoleucine	6.6
11	L-Phenylalanine	4.2
12	L-Tyrosine	9.1
13	L-Serine	11.0
14	L-Glycine	3.74
15	L-Cysteine	0.3
16	L-Histidine	4.24
17	L-Methionine	0.0


Figure 1: Peak Display of TLC-Flavonoid Test of *Sterculia tragacantha* Fruit

### Fatty Acid Profile of Methanol Extract of *Sterculia tragacantha* fruit

The major fatty acid components of the *Sterculia tragacantha* fruit extract was methyl palmitate and methyl tricosanoate (Table 4).

Methyl palmitate can reduce phagocytosis<sup>15</sup>. Methyl palmitate and tiron show a promising Hepatoprotective effect to acetaminophen-induced acute liver injury through inflammatory response modulation and oxidative stress reduction, allowing preservation of liver function<sup>16</sup>. Methyl palmitate and ethyl palmitate are natural fatty acid esters reported as inflammatory cell inhibitors. Methyl palmitate can inhibit some models of inflammatory disorders such as leg edema in mice, lipopolysaccharide-induced endotoxemia in mice and lipopolysaccharide-induced acute lung injury in mice and oil-induced ear edema in mice<sup>17</sup>. Furthermore, MP and EP can reduce neutrophil infiltration in mice. Methyl palmitate has powerful and useful antioxidant, anti-inflammatory and anti-fibrotic properties in silicosis cases<sup>18</sup>.

### Amino Acid Profile of *Sterculia tragacantha* fruit Extract

The main composer of amino acids contained in the extract of *Sterculia tragacantha* fruit are L-Arginine (185.58 µg/gram), L-Proline (58.06 µg/gram), L-Glutamic acid (38.32 µg/gram), L-Valine (34.97 µg/gram), L-Lycine (34.97 µg/gram) dan L-Alanine (31.23 µg/gram) (Table 5).

L-Arginine is effective in improving glomerular entoheliosis in pre eclamptic mouse models<sup>19</sup>. Provision of L-Arginine has been shown to increase endothelial damage to the rat placenta (*Mus musculus*) of the preeclampsia model<sup>20</sup>. Moreover, the administration of L-arginine can cause hepatocyte cell damage in mice (*Mus musculus*) of preeclampsia models assessed from a decrease in liver histopathologic scoring<sup>21</sup>. The effectiveness of L-Arginine treatment is proven to be significant in repairing of Arteri Spiralis damage (tunica intima hyperplasia and also atherosclerosis) in mouse models of preeclampsia (*Mus musculus*)<sup>22</sup>.

The most abundant amino acids are aspartic acid, leucine, glutamic acid and proline in *Sterculia setigera*; aspartic acid, valine, leucine, proline and serine in *Sterculia urens*; aspartic acid, valine, proline, glutamic acid and glycine in *Sterculia villosa*<sup>23</sup>. The major amino acids in defatted *Sterculia urens* cotyledon flour (DSCF) were determined as glutamic acid,

arginine and aspartic acid. Cysteine, methionine, tyrosine and histidine were observed in minor quantities<sup>24</sup>.

## CONCLUSION

Simplicia of *Sterculia tragacantha* fruit contains phytochemical compounds, namely flavonoid, tannin, hydrolyzable tannin, phenolic, poly phenol, and saponin. The methanol extract of papaya woton fruit has a strong antioxidant activity while that n-hexane extract has a potential as antibacterial to *Vibrio alginolyticus*. The main fatty acids of papaya woton fruit extract (*Sterculia tragacantha*) were methyl palmitate and methyl tricosanoate while the main amino acids are L-Arginine, L-Proline, L-Glutamic acid, L-Valine, L-Lycine and L-Alanine.

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