RESEARCH ARTICLE

Phytochemical and antioxidant activity evaluation of the bark of Tampoi (Baccaurea macrocarpa) [version 1; peer review: 3 approved with reservations, 1 not approved]

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Abstract

Background: Tampoi (Baccaurea macrocarpa) is a tropical rainforest plant that produces edible fruit and is native to Southeast Asia, especially East Kalimantan, Indonesia. Previous research showed that Tampoi potentially can be developed as a drug. It was reported that the extract of Tampoi fruit displayed antioxidant activity, which was correlated with its phenolic and flavonoid substances. There is no information about the antioxidant activity of other parts of this plant, such as the bark, which might also have this kind of activity. Therefore, the aim of this study was to evaluate the phytochemical, toxicity, and antioxidant activity of the bark of Tampoi.

Methods: The bark of Tampoi was extracted with methanol and concentrated using rotary evaporator to obtain the methanol extract of the bark. Secondary metabolites of this extract was determined using phytochemical analysis. Afterward, the methanol extract was tested for its toxicity using brine shrimp lethality test and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl method.

Results: Phytochemical evaluation results showed that the methanol extract of bark of this plant contains several secondary metabolites including alkaloids, flavonoids, phenolics, steroids, and triterpenoids. The toxicity test displayed no toxic property due to a LC₅₀ value above 1000 ppm. For antioxidant activity, the result exhibited that the methanol extract of bark of this plant could be categorized as an active extract with IC₅₀ value of 11.15 ppm. Moreover, based on gas chromatography-mass spectrometer analysis, there are 37 isolated compounds from the bark, one of which is methylparaben, a phenolic predicted to act as an antioxidant.

Conclusion: The results obtained in this research demonstrated that the bark of Tampoi (B. macrocarpa) has potential as an antioxidant.

Keywords
Tampoi, Baccaurea macrocarpa, toxicity, BSLT, antioxidant, DPPH
Introduction
Indonesia is a mega-diverse country in terms of biodiversity that is flanked by the Indian and Pacific Oceans. Indonesia’s biodiversity encompasses the diversity of living things both on land and sea\(^1\). Indonesia, especially East Kalimantan, has very extensive tropical rainforest, which is a habitat for much biodiversity. Various types of plants have long been utilized by the community as traditional medicines. The utilization of natural products as an alternative medicine is increasing because natural ingredients are believed to be safer than synthetic substances, i.e. do not contain chemicals that only can be found in modern medicines, which are linked to toxicity\(^7\).

Among plants, the genus of *Baccaurea* have interesting biological activities. For example, *B. angulata* has been reported as a potential functional food with effective antioxidant\(^7\), anti-inflammatory, anti-atherogenic, and hypcholesteremia activities\(^4\). Other research has also investigated the biological activity of other species of this genus, i.e. *B. lanceolata* and *B. macrocarpa*. It was reported that the fruits of *B. macrocarpa* exhibited the highest antioxidant activity compared with *B. lanceolata*, which significantly correlated with the phenolic and flavonoid contents\(^7\).

*B. macrocarpa* is one of the typical plants of East Kalimantan, Indonesia and the edible fruits is a source of additional nutrients and known as Tampoi. Until now, the information about the antioxidant activity of other parts of this plant such as the bark of Tampoi has not been reported yet. Hence, the present research was conducted to investigate the phytochemical, toxicity, and antioxidant activity of the bark of Tampoi (*B. macrocarpa*). Furthermore, the gas chromatography-mass spectrometer (GC-MS) analysis was performed to obtain information about the kinds of isolated compounds contained.

Methods

Extraction
Extraction was carried out as described previously by Erwin *et al.* (2014)\(^4\). The bark of Tampoi (*B. macrocarpa*) was dried for 1 week at room temperature and ground to a powder. The powder was extracted using a maceration method by soaking in methanol for 24 hours at room temperature, which was repeated three times. Afterwards, the extract solution was filtered by filter paper and the solvent was evaporated under vacuum using a rotary evaporator (Buchi R II) at 45°C and 1 atm, to obtain the methanol extract of bark of Tampoi.

Phytochemical evaluation
Phytochemical evaluation was performed to investigate the secondary metabolites contents of the methanol extract of bark of Tampoi (*B. macrocarpa*), including alkaloids, flavonoids, phenolics, steroids, triterpenoids, and saponins, as described previously\(^1\). The presence of secondary metabolites were identified by observing the changing color of the extract. These evaluations were performed as follows:

Alkaloids. 1 mg of extract was inserted into a test tube and then dissolved in 1 mL methanol. A few drops of 1% FeCl\(_3\) were added. Afterwards, a few drops of Dragendorff reagent was added into the mixture. The formation of orange on filter paper indicated the presence of alkaloids.

Flavonoids. 1 mg of extract was inserted into a test tube and diluted in 1 mL methanol. A few 2 mg of Magnesium powder was added followed by a few drops of concentrated HCl. The presence of flavonoids was identified by the formation of pink or red color.

Phenolics. 1 mg of extract was introduced into a test tube and dissolved in methanol. Then a few drops of 1% FeCl\(_3\) were inserted. The formation of green, red, purple, dark blue or black indicated the presence of phenolics.

Steroids and triterpenoids. 1 mL of methanol and 1 mg of extract were inserted into a test tube, stirred until homogeneous, then 2 drops of anhydride acetate and 1 drop of H\(_2\)SO\(_4\) were added (Liebermann Burchard reagent). The formation of green or purple precipitation showed a sample containing steroids, and red precipitation displayed the presence of terpenoids.

Saponins. 1 mg extract was put into a test tube and then dissolved in distilled water, and shaken strongly. The presence of saponins is characterized by the formation of durable foam on the surface of the liquid. Foam that remains stable after the addition of a few drops of concentrated HCl indicated the presence of saponins.

Toxicity test
The toxicity test of extract was performed using brine shrimp lethality test (BSLT), as described previously\(^4\). Methanol extract of bark of Tampoi (*B. macrocarpa*) (1 mg) was dissolved using 100 µL of 1% DMSO (dimethyl sulfoxide) and homogenized. The samples were diluted using 150 µL of distilled water until the total of volume reached 250 µL, and then pipetted 200 µL and diluted again using 600 µL of distilled water until the total of volume was 800 µL, so that the sample concentration was 1000 ppm. Samples with a concentration of 500, 250, 125, 62.5, 31.2, 15.6, and 7.8 ppm were made from sample dilutions of a concentration of 1000 ppm. The control solution was made with the same treatment as the sample without the addition of extract.

The toxicity test was carried out using several standard microplates. About 100 µL seawater containing 8-13 shrimp larvae was added to each diluted sample so that the sample volume was 200 µL (with a concentration of 500, 250, 125, 62.5, 31.2, 15.6, and 7.8 ppm). The number of dead shrimp larvae was calculated for 24 hours after treatment. Each sample was treated in triplicate. The data obtained was recorded and the value of LC\(_{50}\) calculated (Lethal Concentration 50%) using SAS Probit analysis.

Antioxidant assay
The antioxidant activity of the extract was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method, as described previously\(^9\). Briefly, the extract of bark of Tampoi (*B. macrocarpa*) was prepared in a solution with a concentration of 25, 50, 75 and 100 ppm, respectively. 1 mL of extract and 1 mL of DPPH (0.024 mg/mL) were put into a test tube, which was incubated for 30 min at 37°C before being
measured by Spectrophotometer UV Thermo Scientific Evolution 201 (measurements were carried out at a wavelength of 515 nm). Vitamin C was used as a positive control with variations in concentration were 2, 4, 6, and 8 ppm, respectively. Determination of antioxidant activity or DPPH scavenging effect (%) of extract and vitamin C were carried out in triplicate using equation as follow.

\[
\text{percentage of antioxidant activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%.
\]

Then, the value of IC\(_{50}\) (Inhibitory Concentration 50%) was determined using linear regression.

**GC-MS analysis**

In order to obtain the information of the kinds of compounds in methanol extract of bark of Tampoi, an analysis using GC-MS 5977 was performed. Specification of column that used in this research was HP-5MS with length 30 m, diameter 0.25 mm, thick of film 0.25 µm. The identification of the compound was compared to NIST standard data (https://webbook.nist.gov).

**Results**

The secondary metabolites found in the methanol extract of the bark of Tampoi (\textit{B. macrocarpa}) are presented in Table 1.

To evaluate the antioxidant activity of the methanol extract of the bark, DPPH method was performed. The results of the antioxidant test can be seen in Table 2.

Furthermore, the methanol extract was analyzed using GC-MS analysis. The chromatogram and compound contents of this extract is shown in Figure 1 and Table 3, respectively.

**Discussion**

Based on the phytochemical evaluation, the results showed that the methanol extract of bark of Tampoi (\textit{B. macrocarpa}) contains several secondary metabolites including alkaloids, flavonoids, phenolics, steroids, and triterpenoids. Several secondary metabolites including alkaloids, steroids, triterpenoids, flavonoids, and phenolics are known to have antioxidant properties. These antioxidant compounds wield their activities through different mechanisms, for example by inhibiting hydrogen abstraction, radical scavenging, binding transition metal ions, disintegrating peroxides\(^{12,13}\), and one of the most important factors influencing antioxidant activity is the ability of the compounds to donate electrons.

Furthermore, in the present study the antioxidant activity of the Tampoi extract was determined by DPPH method. This method was used because it is simple, efficient, quick, more practical, and relatively inexpensive\(^{14}\). Based on Table 2, it is known that the methanol extract of bark of Tampoi (\textit{B. macrocarpa}) can be categorized as an active extract in an antioxidant assay with

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**Table 1. Phytochemical evaluation of the methanol extract of bark of Tampoi (\textit{Baccaurea macrocarpa}).**

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) Presence; (-) Absence

**Table 2. Antioxidant activity of the methanol extract of bark of Tampoi (\textit{Baccaurea macrocarpa}).** Average of three replicates performed for each concentration.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (ppm)</th>
<th>Absorbance</th>
<th>Inhibition</th>
<th>Percentage of inhibition (%)</th>
<th>IC(_{50}) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sample</td>
<td>Blank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bark</td>
<td>20</td>
<td>0.2190</td>
<td>0.4150</td>
<td>0.47229</td>
<td>47.229</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.0560</td>
<td>0.88193</td>
<td>88.193</td>
<td>11.15</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.0490</td>
<td>0.86506</td>
<td>86.506</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.0305</td>
<td>0.92651</td>
<td>92.651</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>2</td>
<td>0.5470</td>
<td>0.6700</td>
<td>0.18360</td>
<td>18.360</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.1530</td>
<td>0.77160</td>
<td>77.160</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.0450</td>
<td>0.93280</td>
<td>93.280</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.0340</td>
<td>0.94930</td>
<td>94.930</td>
<td></td>
</tr>
</tbody>
</table>
IC₅₀ value of 11.15 ppm. In addition, the results of the toxicity test using the BSLT method showed that the extract was toxic because it displayed LC₅₀ value above 1000 ppm.

According to the results of GC-MS analysis, the chromatogram showed 37 peaks (compounds). The profile of the compounds showed that the main components were fatty acids and fatty acid esters. Total content of unsaturated fatty acids and esters with a peak area of 12.15% including 9,12-octadecadienoic acid (Z,Z)-, methyl ester (peak area 0.04), 9-octadecenoic acid, methyl ester (peak area 8.46), undec-10-yneic acid, undecyl ester (peak area 3.58), undec-10-yneic acid, undecyl ester (peak area 3.346), cis-vaccenic acid (peak area 0.07), and oleic acid (peak area 0.19). It was been reported that unsaturated fatty acid compounds and unsaturated fatty acid esters have significant antioxidant properties\(^{15-17}\).

It can be seen that only a small part of those are aromatic compounds. However, aromatic compounds are compounds that have the ability to stabilize high free radicals. The mechanism of phenolics as antioxidants is started by the formation a bond between free radical (DPPH radical) and hydrogen atom from OH-phenolics (ArOH) to form ArO radical. Hydrogen atom will easier to be released because of the presence of electron withdrawing group which is bound at ortho- or para- positions\(^{18}\). Furthermore, ArO will react with a radical (ArO or other radical) to form a stable compound\(^{19,20}\).

\[
\text{DPPH} + \text{AOH} \rightarrow \text{DPPH-H} + \text{ArO}
\]
\[
\text{DPPH} + \text{ArO} \rightarrow \text{DPPH-OAr} \text{ or DPPH} + \text{R} \rightarrow \text{DPPH-R}
\]

According to identification of the compound in the methanol extract of bark of *Tampoi (Baccaurea macrocarpa)* using NIST database (DRUGBANK accession number, DB14212), it is known that the compound is identified as methylparaben. Based on the NIST database, peak at retention time at 9.467 min and peak area of 0.76% showed the characteristic of methylparaben (Molecular formula=C\(_8\)H\(_8\)O\(_3\); Molecular weight=152).

It has been reported that methylparaben does not show negative effects on male mouse reproduction\(^{21}\). Methylparaben is widely used as a preservative in cosmetic products, medicines or pharmaceutical products and food ingredients\(^{22,23}\), and the antibacterial activity of methylparaben is stronger than benzoate acid\(^{24}\).

Methylparaben is a phenolic group that can reduce free radicals because it contains aromatic groups, -OH clusters and carbonyl groups. The presence of –COOC\(_3\) substituent at para- position in methylparaben makes this compound act as an electron withdrawing group. The bond dissociation energy (BDE) of the O–H bond is a main factor to investigate the action of antioxidant, due to the weaker OH bond the reaction of the free radical will be easier\(^{19}\). As the prediction of the previous reaction mechanism\(^{19}\), the prediction of the reaction mechanism between DPPH radical and methyl paraben can be seen in Figure 2.

Figure 1. GC chromatogram of methanol extract of bark of *Tampoi (Baccaurea macrocarpa)*.
Table 3. Composition of compounds from methanol extract of bark of Tampoi (B. macrocarpa).

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention Time (min)</th>
<th>% Peak Area</th>
<th>Molecule Formula</th>
<th>Molecular Weight</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.467</td>
<td>0.76</td>
<td>C_8H_8O_3</td>
<td>152</td>
<td>Methylparaben</td>
</tr>
<tr>
<td>2</td>
<td>14.877</td>
<td>1.32</td>
<td>C_{14}H_{26}</td>
<td>194</td>
<td>Cyclohexane, 1-(cyclohexylmethyl)-2-methyl-, cis</td>
</tr>
<tr>
<td>3</td>
<td>19.329</td>
<td>0.91</td>
<td>C_{17}H_{34}O_2</td>
<td>270.</td>
<td>Methyl palmitate</td>
</tr>
<tr>
<td>4</td>
<td>20.034</td>
<td>16.14</td>
<td>C_{16}H_{32}O_2</td>
<td>256</td>
<td>palmitic acid</td>
</tr>
<tr>
<td>5</td>
<td>20.227</td>
<td>0.72</td>
<td>C_{16}H_{32}</td>
<td>256</td>
<td>palmitic acid</td>
</tr>
<tr>
<td>6</td>
<td>20.300</td>
<td>3.08</td>
<td>C_{34}H_{65}F_3O_2</td>
<td>562</td>
<td>Dotriacontyl trifluoroacetate</td>
</tr>
<tr>
<td>7</td>
<td>20.432</td>
<td>16.14</td>
<td>C_{34}H_{65}F_3O_2</td>
<td>562</td>
<td>Tricosyl trifluoroacetate</td>
</tr>
<tr>
<td>8</td>
<td>21.234</td>
<td>1.40</td>
<td>C_{18}H_{36}O_2</td>
<td>284</td>
<td>Methyl 7-methylhexadecanoate</td>
</tr>
<tr>
<td>9</td>
<td>21.345</td>
<td>0.72</td>
<td>C_{16}H_{32}O_2</td>
<td>294</td>
<td>9,12-Octadecadienoic acid (Z,Z)-, methyl ester</td>
</tr>
<tr>
<td>10</td>
<td>22.597</td>
<td>0.72</td>
<td>C_{16}H_{32}O_2</td>
<td>296</td>
<td>9-Octadecenoic acid, methyl ester</td>
</tr>
<tr>
<td>11</td>
<td>22.811</td>
<td>0.62</td>
<td>C_{29}H_{56}O_2</td>
<td>424</td>
<td>Eicosyl nonyl ether</td>
</tr>
<tr>
<td>12</td>
<td>23.069</td>
<td>7.05</td>
<td>C_{17}H_{34}O_2</td>
<td>298</td>
<td>Heptadecanoic acid, 16-methyl, methyl ester</td>
</tr>
<tr>
<td>13</td>
<td>23.334</td>
<td>3.34</td>
<td>C_{19}H_{36}O_2</td>
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<td>Undec-10-ynoic acid, undecyl ester</td>
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<tr>
<td>14</td>
<td>23.431</td>
<td>0.29</td>
<td>C_{19}H_{36}O_2</td>
<td>264</td>
<td>9,17-Octadecadienial, (Z)-</td>
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<tr>
<td>15</td>
<td>23.485</td>
<td>0.07</td>
<td>C_{29}H_{56}O_2</td>
<td>396</td>
<td>cis-Vaccenic acid</td>
</tr>
<tr>
<td>16</td>
<td>23.730</td>
<td>1.19</td>
<td>C_{19}H_{36}O_2</td>
<td>282</td>
<td>Oleic Acid</td>
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<tr>
<td>17</td>
<td>23.774</td>
<td>1.15</td>
<td>C_{19}H_{36}O_2</td>
<td>220</td>
<td>(2S,3S,6S)-6-Isopropyl-3-methyl-2-(prop-1-en-2-yl)-3-vinylcyclohexan one</td>
</tr>
<tr>
<td>18</td>
<td>23.794</td>
<td>0.78</td>
<td>C_{23}H_{48}</td>
<td>208</td>
<td>7-Pentadecyne</td>
</tr>
<tr>
<td>19</td>
<td>24.592</td>
<td>0.67</td>
<td>C_{19}H_{36}ClO_2</td>
<td>318</td>
<td>2- Chloropropionic acid, pentadecyl ester</td>
</tr>
<tr>
<td>20</td>
<td>26.520</td>
<td>2.77</td>
<td>C_{22}H_{44}O_2</td>
<td>326</td>
<td>Methyl 18-methyleneadecanoate</td>
</tr>
<tr>
<td>21</td>
<td>26.733</td>
<td>3.58</td>
<td>C_{22}H_{44}</td>
<td>282</td>
<td>Eicosane</td>
</tr>
<tr>
<td>22</td>
<td>27.207</td>
<td>0.87</td>
<td>C_{25}H_{50}F_2O_2</td>
<td>662</td>
<td>Dotriacontyl heptafluorobutrate</td>
</tr>
<tr>
<td>23</td>
<td>27.255</td>
<td>0.08</td>
<td>C_{25}H_{50}Br_2</td>
<td>917</td>
<td>Tetrapentacontane, 1,54-dibromo-</td>
</tr>
<tr>
<td>24</td>
<td>28.234</td>
<td>0.74</td>
<td>C_{23}H_{48}</td>
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<td>Octacosane</td>
</tr>
<tr>
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<td>28.286</td>
<td>1.48</td>
<td>C_{23}H_{48}</td>
<td>659</td>
<td>Pentatriacontane, 13-docosenyiidene-</td>
</tr>
<tr>
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<td>28.374</td>
<td>2.31</td>
<td>C_{21}H_{46}</td>
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<td>1H-Indene, 5-butyl-6-hexyloctahydro-</td>
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<td>28.403</td>
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<td>C_{22}H_{48}F_2O_2</td>
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<td>Nonadecyl trifluoroacetate</td>
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<tr>
<td>28</td>
<td>28.941</td>
<td>1.68</td>
<td>C_{22}H_{44}</td>
<td>400</td>
<td>Nonacos-1-ene</td>
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<tr>
<td>29</td>
<td>28.963</td>
<td>0.31</td>
<td>C_{22}H_{48}F_2O_2</td>
<td>394</td>
<td>Eicosyl trifluoroacetate</td>
</tr>
<tr>
<td>30</td>
<td>28.980</td>
<td>0.34</td>
<td>C_{23}H_{48}F_2O_2</td>
<td>322</td>
<td>9-Tricosene, (Z)-</td>
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<td>31</td>
<td>29.192</td>
<td>1.32</td>
<td>C_{23}H_{44}</td>
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<td>1-Octadecene</td>
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<tr>
<td>32</td>
<td>29.224</td>
<td>1.10</td>
<td>C_{23}H_{46}</td>
<td>364</td>
<td>1-Hexacosene</td>
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<td>33</td>
<td>29.708</td>
<td>7.09</td>
<td>C_{26}H_{54}O_2</td>
<td>354</td>
<td>Methyl 20-methyl-heneicosanoate</td>
</tr>
<tr>
<td>34</td>
<td>29.829</td>
<td>0.10</td>
<td>C_{29}H_{56}</td>
<td>252</td>
<td>1-Octadecene</td>
</tr>
<tr>
<td>35</td>
<td>29.878</td>
<td>0.29</td>
<td>C_{29}H_{56}</td>
<td>400</td>
<td>Nonacos-1-ene</td>
</tr>
<tr>
<td>36</td>
<td>29.907</td>
<td>0.28</td>
<td>C_{29}H_{52}</td>
<td>490</td>
<td>17-Pentatriacontene</td>
</tr>
</tbody>
</table>
Conclusion
The results of the study showed that the bark of Tampoi (Baccaurea macrocarpa) has antioxidant activity with an IC₅₀ value of 11.15 ppm.

Data availability
F1000Research: Dataset 1. Sheet 1, raw data of the results of phytochemical evaluation for alkaloids, flavonoids, phenolics, steroids, triterpenoids, and saponins by observing the changing of colors; Sheet 2, raw data of the observation of the mortality numbers of Artemia salina Leach and calculation of LC₅₀ value in toxicity test using brine shrimp lethality test; Sheet 3, raw data for antioxidant activity by DPPH method, including the measurement of absorbance using spectrophotometer in triplicate, the calculation of percentage of antioxidant activity, and the value of IC₅₀; Sheet 4, raw data of GC-MS analysis. https://doi.org/10.5256/f1000research.16643.d227222

Grant information
The authors acknowledge funding from the Islamic Development Bank (IsDB) project in the frame of Hibah Penelitian PIU IDB for Lecturer Mulawarman University 2018 Number: 2248/UN17.11/PL/2018.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
The authors would like to thank the IsDB project for providing financial support and Head of Plant Anatomy and Systematic Laboratory of Biology Department, Faculty of Mathematics and Natural Sciences of Mulawarman University for identification the specimen.

References

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Version 1

Reviewer Report 15 November 2019

https://doi.org/10.5256/f1000research.18189.r56216

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This manuscript reported the new information of phytochemical and antioxidant activity of barks of *Baccaurea macrocarpa*.

The authors used only DPPH assay to evaluate antioxidant activity. Antioxidant activity should be investigated using various assays to present antioxidant capacity of the extracts.

The results of evaluation on phytochemicals of barks of *B. macrocarpa* showed that the methanol extract consisted of alkaloids, steroids, triterpenoids, flavonoids and phenolic compounds. But the profile of GC-MS showed only fatty acids, fatty acid esters and methyl paraben. The authors should use LC-MS to investigate chemical constituents of methanol extract instead of GC-MS.

In results section, the authors did not mention on the toxicity test of the extract using brine shrimp lethality test. And the results should express yours statistical analysis.

In discussion section, the third paragraph, the authors need to rewrite the total content and composition of fatty acids. GC chromatogram in Figure 1 was not related to the data of composition of compounds in Table 3 such as retention time, % peak area. For example, peak at retention time 19.329 showed high intensity on GC chromatogram but it expressed low % peak area just 0.91. The peak at retention time 14.877 showed low intensity on GC chromatogram but it expressed % peak area 1.32. Moreover, some compounds presented low matching percentage from the library searching. In Figure 2, structure of methyl paraben was wrong.

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

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

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:** Natural Product Chemistry.

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, however I have significant reservations, as outlined above.

Reviewer Report 11 November 2019

[https://doi.org/10.5256/f1000research.18189.r56219](https://doi.org/10.5256/f1000research.18189.r56219)

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Agustono Wibowo
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**Introduction:**
1. In first paragraph line 5, please correct your statement on natural ingredients “do not contain chemicals” that only can be found in modern medicines, as all things in this world is formed from chemical constituents. Please change to “contain toxic chemicals”.

2. Last paragraph line 5, the statement “kinds of isolated compounds contained” are not correct as you don’t isolate the compound. Please remove the word “isolated”.

**Methods:**
1. DDPH assay alone can’t express the antioxidant properties of sample, so we suggest you to add other antioxidant assay such as ABTS and FRAP.

**Discussion:**
1. GCMS result indicated that the main constituent in *Baccaurea macrocarpa* extract is fatty acid, this is because the GCMS can only detect the volatile compounds. To identify other compounds that are responsible in the antioxidant activity of *Baccaurea macrocarpa*, we suggest you to run your
sample using LCMS.

2. Methylparaben is familiar compound. Can you give literature which supported your claim that methylparaben is responsible to the antioxidant of Baccaurea macrocarpa extract?

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?
Partly

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

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

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

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Natural Product Chemistry and Organic Synthesis

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, however I have significant reservations, as outlined above.

Reviewer Report 06 November 2019
https://doi.org/10.5256/f1000research.18189.r56217

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Natthida Weerapreeyakul
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According to the GC chromatogram the peak that was claimed to be methyl paraben was detected at 9.479 min but when identified with the MS the peak at 9.467 min was identified instead. This might be wrong interpretation.

Why the peak with high intensity detected at 19.329 min of methyl palmitate was not considered as the major compound or whether it was contributed to the antioxidant effect?
Due to there are many antioxidant mechanisms, therefore, only DPPH scavenging activity is not sufficient. Cytotoxicity result was not shown and sufficiently discussed in correlation with the antioxidant activity. The statistical analysis should be mentioned in the method section. Based on the insufficient information and evidence, this manuscript needs more experimentation and well written regarding the method, results and discussion.

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
No

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

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacology, Biomedical sciences

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 04 November 2019
https://doi.org/10.5256/f1000research.18189.r44034

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The study design and the methodology sounds good. Still more details on the plant B. macrocarpa need to be included in the introduction. Even though the authors try to substantiate Methyl paraben as a non-toxic compound, it is a universally known fact usage of Methyl paraben is strongly discouraged in all
human usage food and cosmetics as preservative. The Environmental Working Group (EWG) lists methylparaben as being a low to moderate Health Hazard. Parabens are potential endocrine disruptors due to their ability to mimic estrogen. Studies demonstrate that at sufficient concentrations, parabens can increase cell proliferation in human breast cancer MCF-7 cells, which are often used as a sensitive measure of estrogenic activity. Applying personal care product containing parabens—especially methylparaben—can lead to UV-induced damage of skin cells and disruption of cell proliferation (cell growth rate). These are evidenced reports on the Methyl paraben. Nevertheless, it is available in the natural source from the plant in meagre quantity. The authors can check for other compounds in GC-MS and state its importance in the manuscript. The GC-MS can be repeated. or HPLC can be performed using an aqueous extract. A simple TLC becomes handy for compound prediction, Then a column chromatography will be useful to check if there are any useful compounds.

The authors fail to include the ill effects of Methyl paraben in the literature. Is there any reason for avoiding such inclusions? The authors should weigh the importance of other compounds in the plant. Is there any traditional/ancient usage of the fruit mentioned in literature must be included in Introduction section.

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?
Partly

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

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

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:** Plant Phyto chemistry

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, however I have significant reservations, as outlined above.
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