GC-MS profiling and DPPH radical scavenging activity of the bark of Tampoi (Baccaurea macrocarpa) [version 2; peer review: 1 approved, 2 approved with reservations, 1 not approved]

Previously titled: Phytochemical and antioxidant activity evaluation of the bark of Tampoi (Baccaurea macrocarpa)

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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 using GC-MS analysis, toxicity against Artemia salina, and antioxidant activity with DPPH radical scavenging method 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

This article is included in the ICTROS 2018 collection.

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**Author roles:**

- **Erwin E**: Conceptualization, Data Curation, Formal Analysis, Methodology, Writing – Original Draft Preparation; **Pusparohmana WR**: Formal Analysis, Investigation, Methodology; **Sari IP**: Formal Analysis, Investigation, Methodology, **Hairani R**: Investigation, Methodology, Writing – Original Draft Preparation; **Usman U**: Conceptualization, Formal Analysis, Investigation, Methodology

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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. 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. contain toxic chemicals that only can be found in modern medicines, which are linked to toxicity.

Among plants, the genus of *Baccaurea* have interesting biological activities and bark, fruits and leaves of several species are used for medicine such as *B. motleyana* (Rambai) for stomach-ache and sore eyes, *B. brevipes* for the regulation of menstruation, and *B. lanceolata* against stomach-ache. The *B. angulata* has been reported as a potential functional food with effective antioxidant, anti-inflammatory, anti-atherogenic, and hypcholesterolemia activities. 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.

The *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. Tampoi fruit skin 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. contain toxic chemicals that only can be found in modern medicines, which are linked to toxicity.

Methods

Extraction
Extraction was carried out as described previously by Erwin et al. (2014). The bark of Tampoi (*B. macrocarpa*) was dried for one 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. The presence of secondary metabolites was 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 diluted in 1 mL methanol. Then a few drops of HSO4 was added. Afterwards, a few drops of Dragendorff reagent was added into the mixture. The formation of orange color on filter paper indicated the presence of alkaloids.

Phenolics. 1 mg of extract was introduced into a test tube and dissolved in methanol. Then a few drops of 1% FeCl3 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 H2SO4 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. 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 LC50 calculated (Lethal Concentration 50%) using 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[14,15,16]. 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: 2, 4, 6, and 8 ppm, respectively. Determination of antioxidant activity or DPPH scavenging effect (%) of extract and vitamin C were carried out in triplicates using equation as follow.

\[
\text{percentage of antioxidant activity} = \left( \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \right) \times 100\%
\]

Then, the value of IC50 (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 (B. macrocarpa) are presented in Table 1.

The result of toxicity test against Artemia salina larvae of the methanol extract of bark of Tampoi (B. macrocarpa) can be seen in Table 2.

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 3.

Furthermore, the methanol extract was analyzed using GC-MS analysis. The chromatogram and it compound contents of this extract is shown in Figure 1 and Table 4, respectively.

Discussion

Based on the phytochemical evaluation, the results showed that the methanol extract of bark of Tampoi (B. macrocarpa) contains several secondary metabolites including 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 triplicates, the calculation of percentage of antioxidant activity, and the value of IC50; Sheet 4, raw data of GC-MS analysis.

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 3, it is known that the methanol extract of bark of Tampoi (B. macrocarpa) can be categorized as an active extract in an antioxidant assay with IC50 value of 11.15 ppm. In addition, the results of

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**Table 1. Phytochemical evaluation of the methanol extract of bark of Tampoi (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

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**Table 2.**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Absorbance of blank</th>
<th>Absorbance of sample</th>
<th>Percentage of antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.12</td>
<td>0.08</td>
<td>33.3%</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>0.09</td>
<td>33.3%</td>
</tr>
<tr>
<td>6</td>
<td>0.18</td>
<td>0.08</td>
<td>55.6%</td>
</tr>
<tr>
<td>8</td>
<td>0.20</td>
<td>0.08</td>
<td>50.0%</td>
</tr>
</tbody>
</table>

---

**Figure 1.**

**Table 3.**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Absorbance of blank</th>
<th>Absorbance of sample</th>
<th>Percentage of antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.12</td>
<td>0.08</td>
<td>33.3%</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>0.09</td>
<td>33.3%</td>
</tr>
<tr>
<td>6</td>
<td>0.18</td>
<td>0.08</td>
<td>55.6%</td>
</tr>
<tr>
<td>8</td>
<td>0.20</td>
<td>0.08</td>
<td>50.0%</td>
</tr>
</tbody>
</table>

---

**Dataset 1.**

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 triplicates, 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
Table 2. Toxicity test of methanol extract of bark of Tampoi (B. macrocarpa).

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Log Concentration</th>
<th>Average of total larvae</th>
<th>Average of % Mortality</th>
<th>Probit</th>
<th>LC$_{50}$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>2.6989</td>
<td>10.3</td>
<td>2.3</td>
<td>22.3</td>
<td>4.23</td>
</tr>
<tr>
<td>250</td>
<td>2.3979</td>
<td>10.7</td>
<td>2.7</td>
<td>25.2</td>
<td>4.33</td>
</tr>
<tr>
<td>125</td>
<td>2.0969</td>
<td>10.3</td>
<td>3.3</td>
<td>32.0</td>
<td>4.53</td>
</tr>
<tr>
<td>62.5</td>
<td>1.7959</td>
<td>10.3</td>
<td>1</td>
<td>9.7</td>
<td>3.66</td>
</tr>
<tr>
<td>31.2</td>
<td>1.4948</td>
<td>10</td>
<td>4.3</td>
<td>43</td>
<td>4.82</td>
</tr>
<tr>
<td>15.6</td>
<td>1.1938</td>
<td>9.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7.8</td>
<td>0.8928</td>
<td>9.3</td>
<td>2.7</td>
<td>29</td>
<td>4.45</td>
</tr>
</tbody>
</table>

Table 3. Antioxidant activity of the methanol extract of bark of Tampoi (Baccaurea macrocarpa). Average of three replicates performed for each concentration.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (ppm)</th>
<th>Absorbance Sample</th>
<th>Inhibition</th>
<th>Percentage of inhibition (%)</th>
<th>IC$_{50}$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark</td>
<td>20</td>
<td>0.2190</td>
<td>0.47229</td>
<td>47.229</td>
<td>11.15</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.0560</td>
<td>0.88193</td>
<td>88.193</td>
<td></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>0.18360</td>
<td>18.360</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.1530</td>
<td>0.77160</td>
<td>77.160</td>
<td></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>

Figure 1. GC chromatogram of methanol extract of bark of Tampoi (Baccaurea 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>9.479</td>
<td>0.76</td>
<td>C₈H₁₀O₃</td>
<td>152</td>
<td>Methylparaben</td>
</tr>
<tr>
<td>2</td>
<td>14.877</td>
<td>1.32</td>
<td>C₁₄H₂₆</td>
<td>194</td>
<td>Cyclohexane, 1-(cyclohexylmethyl)-2-methyl-, cis</td>
</tr>
<tr>
<td>3</td>
<td>19.329</td>
<td>9.91</td>
<td>C₁₇H₃₄O₂</td>
<td>270</td>
<td>Methyl palmitate</td>
</tr>
<tr>
<td>4</td>
<td>20.034</td>
<td>16.14</td>
<td>C₁₆H₃₂O₂</td>
<td>256</td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>5</td>
<td>20.227</td>
<td>0.72</td>
<td>C₁₆H₃₂O₂</td>
<td>256</td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>6</td>
<td>20.300</td>
<td>3.08</td>
<td>C₁₆H₃₂F₂O₂</td>
<td>562</td>
<td>Dotriacontyl trifluoroacetate</td>
</tr>
<tr>
<td>7</td>
<td>20.432</td>
<td>3.18</td>
<td>C₁₆H₃₂F₂O₂</td>
<td>562</td>
<td>Tricosyl trifluoroacetate</td>
</tr>
<tr>
<td>8</td>
<td>21.234</td>
<td>1.40</td>
<td>C₁₈H₃₄O₂</td>
<td>284</td>
<td>Methyl 7-methylhexadecanoate</td>
</tr>
<tr>
<td>9</td>
<td>22.481</td>
<td>4.23</td>
<td>C₁₉H₃₈O₂</td>
<td>294</td>
<td>9,12-Octadecadienoic acid (Z,Z)-, methyl ester</td>
</tr>
<tr>
<td>10</td>
<td>22.597</td>
<td>8.46</td>
<td>C₁₉H₃₈O₂</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>C₁₉H₃₈O₂</td>
<td>424</td>
<td>Eicosyl nonyl ether</td>
</tr>
<tr>
<td>12</td>
<td>23.069</td>
<td>7.05</td>
<td>C₂₀H₄₀O₂</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>C₂₀H₄₀O₂</td>
<td>336</td>
<td>Undec-10-ynoic acid, undecyl ester</td>
</tr>
<tr>
<td>14</td>
<td>23.431</td>
<td>0.29</td>
<td>C₂₀H₄₂O₂</td>
<td>264</td>
<td>9,17-Octadecadienal, (Z)-</td>
</tr>
<tr>
<td>15</td>
<td>23.485</td>
<td>0.07</td>
<td>C₂₀H₄₂O₂</td>
<td>396</td>
<td>cis-Vaccenic acid</td>
</tr>
<tr>
<td>16</td>
<td>23.730</td>
<td>1.19</td>
<td>C₂₀H₄₂O₂</td>
<td>282</td>
<td>Oleic Acid</td>
</tr>
<tr>
<td>17</td>
<td>23.774</td>
<td>1.15</td>
<td>C₂₀H₄₂O₂</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>C₂₁H₄₂</td>
<td>208</td>
<td>7-Pentadecyne</td>
</tr>
<tr>
<td>19</td>
<td>24.592</td>
<td>0.67</td>
<td>C₂₁H₄₂ClO₂</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>C₂₁H₄₂O₂</td>
<td>326</td>
<td>Methyl 18-methylnonadecanoate</td>
</tr>
<tr>
<td>21</td>
<td>26.733</td>
<td>3.58</td>
<td>C₂₂H₄₄</td>
<td>282</td>
<td>Eicosane</td>
</tr>
<tr>
<td>22</td>
<td>27.207</td>
<td>0.87</td>
<td>C₂₂H₄₄F₂O₂</td>
<td>662</td>
<td>Dotriacontyl heptafluorobutyrate</td>
</tr>
<tr>
<td>23</td>
<td>27.255</td>
<td>0.08</td>
<td>C₂₂H₄₄Br₂</td>
<td>917</td>
<td>Tetrapentaccontane, 1,54-dibromo-</td>
</tr>
<tr>
<td>24</td>
<td>27.374</td>
<td>2.31</td>
<td>C₂₂H₄₂</td>
<td>264</td>
<td>Octacosane</td>
</tr>
<tr>
<td>25</td>
<td>28.234</td>
<td>0.74</td>
<td>C₂₂H₄₄</td>
<td>394</td>
<td>Octacosane</td>
</tr>
<tr>
<td>26</td>
<td>28.286</td>
<td>1.48</td>
<td>C₂₂H₄₂</td>
<td>659</td>
<td>Pentatriacontane, 13-docosenylidene-</td>
</tr>
<tr>
<td>27</td>
<td>28.374</td>
<td>2.33</td>
<td>C₂₂H₄₂</td>
<td>264</td>
<td>1H-Indene, 5-butyl-6-hexyloctahydro-</td>
</tr>
<tr>
<td>28</td>
<td>28.403</td>
<td>2.33</td>
<td>C₂₂H₄₄F₂O₂</td>
<td>380</td>
<td>Nonadecyl trifluoroacetate</td>
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<td>28.941</td>
<td>1.68</td>
<td>C₂₃H₄₄</td>
<td>400</td>
<td>Nonacos-1-ene</td>
</tr>
<tr>
<td>30</td>
<td>28.963</td>
<td>0.31</td>
<td>C₂₃H₄₄F₂O₂</td>
<td>394</td>
<td>Eicosyl trifluoroacetate</td>
</tr>
<tr>
<td>31</td>
<td>28.980</td>
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<td>322</td>
<td>9-Tricosene, (Z)-</td>
</tr>
<tr>
<td>32</td>
<td>29.192</td>
<td>1.32</td>
<td>C₂₃H₄₆</td>
<td>252</td>
<td>1-Octadecene</td>
</tr>
<tr>
<td>33</td>
<td>29.224</td>
<td>1.10</td>
<td>C₂₃H₄₆</td>
<td>364</td>
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<td>34</td>
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<td>7.09</td>
<td>C₂₃H₄₆O₂</td>
<td>354</td>
<td>Methyl 20-methyl-heneicosanoate</td>
</tr>
<tr>
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<td>29.829</td>
<td>0.10</td>
<td>C₂₃H₄₆</td>
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</tr>
<tr>
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<tr>
<td>37</td>
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<td>0.28</td>
<td>C₂₃H₇₀</td>
<td>490</td>
<td>17-Pentatriacontene</td>
</tr>
</tbody>
</table>
the toxicity test using the BSLT method showed that the extract was not 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 19.88% including 9,12-octadecadienoic acid (Z,Z)-, methyl ester (peak area 4.23), 9-octadecenoic acid, methyl ester (peak area 8.46), undec-10-ynoic acid, undecyl ester (peak area 3.58), undec-10-ynoic acid, undecyl ester (peak area 3.346), cis-vaccenic acid, methyl ester (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.

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. Furthermore, ArO will react with a radical (ArO or other radical) to form a stable compound.

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

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

According to identification of the compound in the methanol extract of bark of *Tampoi* (*B. 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 9.479 min and peak area of 0.76% showed the characteristic of methylparaben (Molecular formula=C14H22O3; Molecular weight=152).

Methylparaben is widely used as a preservative in cosmetic products, medicines or pharmaceutical products and food ingredients, and the antibacterial activity of methylparaben is stronger than benzoate acid. Methylparaben does not show negative effects on male mouse reproduction, but it was shown to have androgen antagonistic activity, to act as inhibitors of the sulfotransferase enzyme and to possess genotoxic activity. Paraben is allegedly able to trigger breast cancer in women.

Methylparaben is a phenolic group that can reduce free radicals because it contains aromatic groups, -OH clusters and carbonyl groups. The presence of –COOCH3 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. As the prediction of the previous reaction mechanism, the prediction of the reaction mechanism between DPPH radical and methyl paraben can be seen in Figure 2.

Figure 2. Prediction of DPPH radical scavenging mechanism by methylparaben.
Conclusion
The results of the study showed that the bark of Tampoi (Baccaurea macrocarpa) has antioxidant activity with an IC$_{50}$ 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 LC$_{50}$ 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

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

References
Open Peer Review

Current Peer Review Status: ? × ✔ ?

Version 2

Reviewer Report 20 December 2019

https://doi.org/10.5256/f1000research.23631.r57803

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Chanya Chaicharoenpong
Institute of Biotechnology and Genetic Engineering, Molecular Crop Research Unit, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

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

1. 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.

2. Check the scientific name through the whole manuscript. When the scientific name appears first time in manuscript, it is full written both Genus and species, and then use the abbreviated binomial form for the following times.

3. In methods section, Phytochemical evaluation of alkaloids: “H₂SO₄ 1M” must correct to “1M H₂SO₄”

4. In methods section, Phytochemical evaluation of alkaloids: Please correct your statement “The formation of orange on filter paper....”

5. In methods section, Steroids and triterpenoids: “green or purple precipitation/red precipitation” must correct to “green or purple precipitate/red precipitate”

6. In methods section, the details of GC and MS conditions must clearly clarify.

7. In results section, the results should express your statistical analysis.

8. In results section, Table 2: It is essential to set the concentration of sample for calibration range to less than 50% mortality and greater than 50% mortality, and the calibration curve is linear. The results should express your statistical analysis.
9. In results section, Table 3: Check the data and calculation in Table 3. The results should express your statistical analysis.

10. In results section, Table 4: Data of peak no. 13 does not complete.

11. In discussion section, the data of GC-MS analysis (page 7) does not match the data in Table 4.

12. In discussion section, correct the equation of DPPH and ArOH on page 7.

13. In discussion section, correct “benzoate acid” to “benzoic acid”.

14. To improve the quality of English, the authors must correct grammatical errors and English improvement throughout the manuscript.

**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 16 December 2019

https://doi.org/10.5256/f1000research.23631.r57802

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Natthida Weerapreeyakul
Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand

I respectfully reject this manuscript as following reasons:

This manuscript contains insufficient data although the authors tried to explain that this is a screening study.

In addition to my previous comments I have following notifications:

- Table 2, the LC\textsubscript{50} value which is 1577.89 ppm was not in the concentration range (7.8 - 500 ppm) used in the study.

- Table 3, Inhibition should be rewritten.

- Table 3, an IC\textsubscript{50} value (11.15 ppm) of bark was suspicious as the lowest concentration used was 20 ppm.
There is some typing error of DPPH reaction as AOH supposed to be ArOH.

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

**Reviewer Expertise:** Cancer, Apoptosis, Cell base assay, Anioxidation, Antiproliferation, Chemometric analysis

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.

16 December 2019

Reviewer Report 16 December 2019

https://doi.org/10.5256/f1000research.23631.r57801

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Agustono Wibowo
Faculty Applied Science, Universiti Teknologi MARA Pahang Branch, Jengka Campus, Pahang Darul Makmur, Malaysia

Approved.

**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.

Version 1

Reviewer Report 15 November 2019

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

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Chanya Chaicharoenpong
Institute of Biotechnology and Genetic Engineering, Molecular Crop Research Unit, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
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 comment #1
The authors used only DPPH assay to evaluate antioxidant activity. The antioxidant activity should be investigated using various assays to present antioxidant capacity of the extracts. The results of evaluation on phytochemical 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.

Author response #1
This study is an initial screening of the antioxidant activity of Tampoi bark. The next project if we get the funding, we will use another antioxidant test and other instruments as you suggest. We will also carry out isolation and purification to obtain active compounds from Tampoi bark. Thank you for your valuable suggestion

Reviewer comment #2
In the results section, the authors did not mention on the toxicity test of the extract using brine shrimp lethality test. And the results should express your statistical analysis

Author response #2
Toxicity test data have been added in the revised article

Reviewer comment #3
some compounds presented low matching percentage from the library searching

Author response #3
It has been fixed

Reviewer comment #4
In Figure 2, the structure of methylparaben was wrong.

Author response #4
The structure of methylparaben has been fixed

Reviewer comment #5
In the 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 a 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.

Author response #5
The percentage (%) peak area of GC chromatogram has been fixed

**Competing Interests:** no competing interests
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.

---

**Author Response 21 Nov 2019**

**erwin erwin**, Faculty of Mathematics and Natural Sciences Mulawarman University, Samarinda, Indonesia

Reviewer comments #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”

Author response #1
Thank you for your suggestion, It has been fixed

Reviewer comments #2
The statement “kinds of isolated compounds contained” are not correct as you don’t isolate the compound. Please remove the word “isolated”

Author response #2
The word isolated has been removed

Reviewer comments #3
Methods:
DPPH assay alone can’t express the antioxidant properties of the sample, so we suggest you add other antioxidant assays as ABTS and FRAP

Author response #3
This study is an initial screening of the antioxidant activity of Tampoi bark.

Reviewer comments #4
**Discussion:**
GCMS result indicated that the main constituent in *Baccaurea macrocarpa* extract is a fatty acid, this is because the GCMS can only detect the volatile compounds. To identify other compounds that are responsible for the antioxidant activity of *Baccaurea macrocarpa*, we suggest you to run your sample using LCMS.

Author response #4
The next project if we get the funding, we will use another antioxidant test and other instruments as
you suggest. We will also carry out isolation and purification to obtain active compounds from Tampoi bark.

Reviewer comments #5
Methylparaben is a familiar compound. Can you give literature which supported your claim that methylparaben is responsible for the antioxidant of *Baccaurea macrocarpa* extract?

Author response #5
I could not find in the literature that discusses the antioxidant properties of parabens in vegetation, but methylparaben is preservative and antioxidant in cosmetic products, medicines or pharmaceutical products, and food ingredients. Based on GC-MS data, methylparaben is most likely to be antioxidants

*Competing Interests:* no competing interests

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Natthida Weerapreeyakul
Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand

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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Chinnadurai Immanuel Selvaraj
Department of Biotechnology, School of Biosciences and Technology,, VIT University, Vellore, Tamil Nadu, India

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.

---

**Author Response 21 Nov 2019**

**erwin erwin,** Faculty of Mathematics and Natural Sciences Mulawarman University, Samarinda, Indonesia

**Reviewer comment #1**
The authors can check for other compounds in GC-MS and state its importance in the manuscript

**Author response #1**
We have re-checked the 37 peaks which appeared to be methylparaben most likely to be antioxidants

**Reviewer comment #2**
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.

**Author response #2**
This study is an initial screening of the antioxidant activity of Tampoi bark. The next project if we get the funding, we will use other instruments as you suggested. We will also carry out isolation and purification to obtain active compounds from Tampoi bark. We appreciated your suggestion.
Reviewer comment #3
The authors fail to include the ill effects of Methyl paraben in the literature

Author response #3
We have added additional literature about the side effects of methyl paraben. Ref. no: 27

Reviewer comment #4
Is there any traditional/ancient usage of the fruit mentioned in literature must be included in the Introduction section

Author response #4
We did not find any use of tampoi as traditional medicine, but several other species of the genus Baccarea were used as traditional medicine. In addition, there are several preliminary studies on Tampoi’s bio-activity. Ref. no: 3,4,8, and 9

**Competing Interests:** no competing interests