In vitro antioxidant potential and phytochemical profiling of *Melastoma malabathricum* leaf water extract

Oke Anandika LESTARI1,2, Nurheni Sri PALUPI1,3*, Agus SETIYONO4, Feri KUSNANDAR1,3, Nancy Dewi YULIANA1,5,6

**Abstract**

*Melastoma malabathricum* is one of the medicinal plants plated in West Kalimantan, Indonesia. Its leaves extraction using organic solvents revealed bioactive compounds and antioxidant activity. However, the antioxidant activity, toxicity, and chemical profile of water extract of *M. malabathricum* leaves are still unknown. This study aimed to determine extraction conditions that provide water extract of *M. malabathricum* with the highest antioxidant activity and the lowest toxicity. The extraction conditions were conducted at different water temperatures (25 ± 2 ºC and 90 ± 2 ºC), concentrations (10 and 5 g leaves/100 mL water), and extraction time (15, 30, and 45 minutes). Data analysis was performed by one-way ANOVA followed by Duncan's test (p < 0.05). The result showed that *M. malabathricum* leaves of 5g/100 mL extracted with hot water (90 ± 2 ºC) for 15 minutes showed the highest antioxidant activity (IC50 2.13 ± 19.20 ppm), which was higher than vitamin C (IC50 4.32 ± 0.16 ppm), and low toxicity (LC50 333.06 ± 99.45 ppm). Six compounds namely, 4-O-caffeoylquinic acid, quercimeritin, digiprolactone, 3-O-trans-coumaroylquinic acid, norbergenin, and arteamisin were identified for the first time in the water extract of *M. malabathricum* leaves. These compounds, individually or extracted from other ingredients, have previously been reported to have antioxidant activity.

**Keywords:** antioxidant activity; *Melastoma malabathricum*; phytochemical profile; toxicity; water extract.

**Practical Application:** *M. malabathricum* leaves have antioxidant potential by brewing 5 g of dried leaves using hot water (90 ºC) for 15 minutes. These results can be a practical brewing approach like herbal tea.

1 Introduction

*Melastoma malabathricum* is a medicinal plant from West Kalimantan, Indonesia. It is traditionally used to treat diabetes, diarrhea, dysentery, and high blood pressure (Zheng et al., 2021). The plant has several local names senduduk or halendong (Kumar et al., 2013). The plant leaves are usually boiled with two glasses of water up to half and cooled at room temperature before being consumed as herbal medicine.

Antioxidants are compounds that might donate electrons to free radicals. The body naturally produces free radicals in metabolic processes, and the body has enzymes that act as antioxidants to neutralize. Extreme conditions such as smoking (Śliwińska-Mosson & Milnerowicz 2017), diabetics (Hosseini et al., 2014), and high-intensity exercise (Kawamura & Muraoka 2018) may increase the production of free radicals, thus requiring the intake of antioxidants from external sources. Oxidative stress may lead to many serious diseases such as cancer (Reuter et al., 2010), inflammation (Pashkow, 2011), cardiovascular (D’Oria et al., 2020; Senoner & Dichtl, 2019), and pancreatitis (Robles et al., 2013), which can trigger diabetes (Alizadeh, 2020). The search for new antioxidant sources, especially from the plant, is becoming increasingly important and beneficial. Many plants have been reported to have excellent antioxidant activity, including *Camelina sativa* (Karamać et al., 2020), *Annona muricata* (Orak et al., 2019), *Guazuma ulmifolia* (Rafi et al., 2020), *Shonchus arvensis* (Rafi et al., 2021), *Beta vulgaris* (Gheith & El-Mahmoudy, 2018) and *Syzygium polyanthum* (Syabana et al., 2021). Interestingly, plant with antioxidant activity was also reported to have another bioactivity. For example, *Camelina sativa* was reported to exhibit hypoglycemic and hypolipidemic (Tsykalo & Trzhetsynskyi, 2020), antidiabetic (Chauhan et al., 2010), and anti-inflammatory (Campbell et al., 2010).

The fruit of *M. malabathricum* was recently reported to have the antioxidant capacity and positively correlate with their maturity (Kasunmala et al., 2020). The leaves extract *M. malabathricum* was also revealed anti-nociception activity in vivo (Zakaria et al., 2016) and gastroprotection activity (Zakaria et al., 2015). Despite its common practice to consume this plant as herbal medicine, the reports on the most suitable extraction conditions for *M. malabathricum* to obtain the most optimum antioxidant benefit are still scarce.

**Received 19 Oct., 2021**

**Accepted 01 Feb., 2022**

1 Department of Food Science and Technology, IPB University, Bogor, Indonesia
2 Department of Food Science and Technology, Faculty of Agriculture, Tanjungpura University, Pontianak, Indonesia
3 Southeast Asian Food and Agricultural Science and Technology Center – SEAFAST, IPB University, Bogor, Indonesia
4 Department of Clinic Reproduction and Pathology, IPB University, Bogor, Indonesia
5 Halal Science Center, IPB University, Bogor, Indonesia
6 Tropical Biopharmaca Research Center, IPB University, Bogor, Indonesia

*Corresponding author: hnpalupi@apps.ipb.ac.id*
The methanolic extract of the *M. malabathricum* leaves contained several bioactive compounds. These compounds were ascorbic acid, ellagic acid, b-sitosterol 3-O-b-D-glucopyranoside, 2a-hydroxyursolic acid, glycolipid glycerol, 1,2-dilinolenyl-3-O-b-D-galactopyranoside, kaempferol, kaempferol 3-O-b-D-glucopyranoside, kaempferol 3-O-a-L-rhamnopyranoside, kaempferol 3-O-b-D-galactopyranoside, kaempferol 3-O-(2′,6′-di-O-e-p-coumaryl)-b-D-galactopyranoside, ursolic acid, and quercetin (Wong et al., 2012). Another study showed the leaves extracted with hexane, ethyl acetate, and methanol contained α-amyrin, auranamide, patricscabratine, quercitin, kaempferol-3-O-(2′,6′-di-O-p-trans-coumaryl)-β-glucoside, and quercetin (Sirat et al., 2010). The study on the phytochemical profile of *M. malabathricum* water extract is very limited. This study aimed to obtain the most optimum extraction condition to obtain the highest DPPH antioxidant activity. Several extraction conditions were used, i.e., water temperature (25 ± 2 ºC and 90 ± 2 ºC), concentrations (10 and 5 g leaves/100 mL water), and extraction time (15, 30, and 45 minutes). The toxicity of the extract was also tested using Brine Shrimp Lethality Test (BSLT). Finally, the phytochemical profile of the most potent extract was determined using LC-MS/MS.

2 Materials and methods

2.1 Materials

*M. malabathricum* leaves were obtained from from Rasau Jaya, West Kalimantan, Indonesia, and has been identified by Herbarium Bogoriense, Botanical Research Center for Biology-Indonesia Institute of Sciences.

2.2 Extract preparation

*M. malabathricum* was collected in March 2020 between 06.00-08.00 a.m. The leaves used were the shoots and the top six leaves, then rinsed and drained. The leaves were dried at room temperature until the moisture content was < 10%, measured by the gravimetric method (± 72 hours). This method is suitable to produce a higher antioxidant activity than hot air drying (60 ºC) and sun drying (Lemus-Mondaca et al., 2018). The dried leaves were crushed using a dry blender, then sieved through an 18 mesh sieve. Nine extraction conditions were established (Table 1). The extraction was carried out according to the treatment, then filtered with Whatman filter paper (grade 1) and distilled water added up to 100 mL. The filtrate was concentrated by using a rotary evaporator at 40 ºC. The yield of each treatment was calculated based on the dry weight.

2.3 Antioxidant activity measurement

Antioxidant activity was determined by the scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Chang et al., 2020; Młynarczyk & Walkowiak-Tomczak, 2017). DPPH solution was prepared by dissolve 80 mg DPPH in 100 mL methanol. 2 mL sample was mixed with 2 mL of DPPH solution. Before measuring the absorbance at 517 nm, the mixture was homogenized and incubated for 30 minutes in the dark at room temperature. Antioxidant activity was expressed in IC50 value (ppm). Measurements were done in triplicates. Percentage of antioxidant activity was calculated as follows: inhibition (%) = 1 – (sample absorbance/blank absorbance) × 100%.

2.4 Toxicity assay

Toxicity was measured by the Brine Shrimp Lethality Test (BSLT) (Marzuki et al., 2019). The 48 hours-old *Artemia salina* larvae were used, with hatching conditions in a transparent container and 40-60 watts of lighting to provide a temperature of 25-30 ºC. The sample stock solution was prepared by dissolving 20 mg of sample into 10 mL of seawater (2000 ppm). Vial (2000 μL) contained ten *Artemia salina* larvae (in 1000 μL) was added of 1000, 500, 100, and 10 ρL stock solutions. Observations were maintained for 24 hours by enumerating the number of dead larvae. In addition, probit analysis was carried out to obtain the LC50 value. Measurements were triplicate. The LC50 value is defined as the concentration which causes 50% mortality of larvae, and the linear regression (y = a + bx) was established. Toxicity level was indicated by criteria including very toxic (1-10 ppm), moderately toxic (10-100 ppm), and low toxic (100-1000 ppm) (Meyer et al., 1982).

2.5 Phytochemical profiling using LC-MS/MS

LC-MS/MS measurement was conducted according to Faraone et al. (Faraone et al., 2019) using LC-MS/MS (Waters LC-MS/MS measurement was conducted according to Faraone et al. (Faraone et al., 2019) using LC-MS/MS.

<table>
<thead>
<tr>
<th>No</th>
<th>Code</th>
<th>Water Temperature (ºC)</th>
<th>Concentration (g leaves/100 mL water)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N1015</td>
<td>25 ± 2</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>N1030</td>
<td>25 ± 2</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>N1045</td>
<td>25 ± 2</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>H1015</td>
<td>90 ± 2</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>H1030</td>
<td>90 ± 2</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>H1045</td>
<td>90 ± 2</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>7</td>
<td>H0515</td>
<td>90 ± 2</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>H0530</td>
<td>90 ± 2</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>H0545</td>
<td>90 ± 2</td>
<td>5</td>
<td>45</td>
</tr>
</tbody>
</table>

Note: The first letter of the code indicates the temperature of the water used is normal (N) or hot (H), the first two numbers indicate the concentration of leaves, and the next two numbers indicate the extraction time.

Table 1. Nine extraction conditions of *M. malabathricum* dried powdered leaves.
Xevo G2-X2 QToF). The column Atlantis T3 C18 (100x 2.10 mm; 3.00 m particle size) with Electrospray Ionization (ESI) method was used in positive ionization mode with the mass range of 50 m/z to 1200 m/z. The operational temperature was 40 ºC. The mobile phase was used in gradient, comprising 0.10% formic acid in water (solvent A) and 0.10% formic acid in acetonitrile (solvent B). The following gradient program was performed: 0–1 min 5% B, 1–8 min 40% B, 8–11 min 100% B, 11–13 min 100% B, and 13–16 min 95% B at flow rate 0.30 mL/min. The sample (0.01 g) was dissolved in 1 μL of 100% methanol (LC grade) and diluted ten times. Then, 1 μL of sample were injected. Identification of compounds was validated using UNIFI Scientific library and Chemspider.

### 2.6 Data analysis

Data analysis of extraction yield, antioxidant activity, and toxicity was carried out using SPSS statistical software v.23 with one-way ANOVA, followed by Duncan’s test. The statistically significant result was established at p < 0.05. Compound profiling was analysis and processing by using the UNIFI Scientific Information System.

### 3 Results and discussion

Nine extraction conditions of *M. malabathricum* leaves generated significantly different extraction yields of 5.11 – 21.37% (p < 0.05). It is shown that H1045 (90 ± 2 ºC, 10 g/100 mL, 45 min) and H1030 (90 ± 2 ºC, 10 g/100 mL, 30 min), had the highest extraction yield followed by H0545 (90 ± 2 ºC, 5 g/100 mL, 45 min), and H0530 (90 ± 2 ºC, 5 g/100 mL, 30 min). In contrast, N1015 had the lowest yield (25 ± 2 ºC, 10 g/100 mL, 15 minutes) (Figure 1).

Based on these results, the higher water temperature with the same extraction time and concentration resulted in a higher yield. Lower leaf concentrations at the same extraction time but using hot water, resulted in the higher the yield. Additionally, a higher water temperature may aid the extraction process. It was reported that high temperature elevates the dissolving ability of analytes (Vergara-Salinas et al., 2012). An increase in extraction yield, was also observed with an increase of extraction time. There was no significant (P > 0.05) difference between extraction yield obtained from 30 and 45 minutes at the same concentration. The results of previous studies on green tea extraction showed that the yield increased by increasing temperature and time, but the highest yield was reached at 95 ºC for 20 minutes (Balci & Özdemir, 2016).

Antioxidant activity decreased with increasing extraction time at 10 g/100 mL concentration with the normal (room) or hot temperature water. In contrary, an increasing extraction time did not give significant difference (P > 0.05) in antioxidant activity at lower concentration (5 g/100mL) with hot water (Figure 2). Low concentrations are thought to result in higher ambient temperatures, thus allowing maximum extraction of components. The results show that the extraction condition takes an optimum time of 15 minutes. Antioxidant activity increased with increasing temperature, but at high temperatures also allows chemical degradation to occur so that it changes the molecular structure that is sensitive to temperature (Chang et al., 2020). Other studies also show that the longer the extraction time, the antioxidant activity increases (Marliani et al., 2017). The ability of the extract to scavenging DPPH radicals depends on the procedure and time of extraction (Nikniaz et al., 2016). Polyphenol is a compound that easily oxidized and converted into quinine or ketone substances that provide DPPH scavenging by hydrogen atom donors (Hou et al., 2016). The phytochemical contained water extract of *M. malabathricum* leaves may provide hydrogen donor by their chemical structure.

![Figure 1](image_url) Extract yield obtained by several extraction conditions. The different letter show the result are significantly different (p < 0.05). Sample code explanation is provided Table 1. The first letter of the code indicates the temperature of the water used is normal (N = 25 ± 2 ºC) or hot (H = 90 ± 2 ºC), the first two numbers indicate the concentration of leaves (g/mL), and the next two numbers indicate the extraction time (min).
Phytochemical and antioxidant activity of Melastoma Malabathricum leaves generated significantly different toxicity with an IC\textsubscript{50} around 333.06 to 613.08 ppm (p < 0.05), which means low toxicity. It is shown the highest toxicity that N1045 (25 ± 2 °C, 10 g/100 mL, 45 minutes), N1030 (25 ± 2 °C, 10 g/100 mL, 30 minutes), H0515 (90 ± 2 °C, 5 g/100 mL, 15 min), and H1045 (90 ± 2 °C, 10 g/100 mL, 45 minutes). In contrast, the lowest toxicity has resulted in H0545 (90 ± 2 °C, 5 g/100 mL, 45 min) (Figure 3).

Brine Shrimp Lethality Test (BSLT) is one of the methods for pre-screening the bioactivity of toxic extracts and determined the LC\textsubscript{50} value. The mortality rate was determined in Artemia salina after 24 hours of sample exposure. This study showed that mortality above 50% occurred at concentrations above 100 ppm, and the mortality rate was directly proportional to the concentration of the extract. The nine extraction conditions of M. malabathricum leaves were significantly different, but all extracts had low toxicity (IC\textsubscript{50} 100 - 1000 ppm). The toxicity test as acute toxicity with experimental animals on M. malabathricum leaves has been carried out in previous studies, which showed that it was not toxic up to a concentration of 2000 ppm in ethanol (Balamurugan et al., 2014) and methanol extract (Kumar et al., 2013).

The extraction conditions of H0515 (90 ± 2 °C, 5 g/100 mL, 15 minutes) are considered as the most ideal conditions, since low concentration and short extraction time gave a higher antioxidant activity as compared to other. This extract was further characterized using LC-MS. Based on the Unifi system and Chemsipder library, the water extract of M. malabathricum leaves (H0515) has contained nine chemical compounds (Table 2).
Chemical compounds in the water extract of *M. malabathricum* leaves are established by the retention time (RT) of 1.3 – 5.52 min (Figure 4). Quercetin is a predominant compound with the highest level of 24.09%, followed by 4-O-caffeoylquinic acid, quercimeritin, digiprolactone, 3-O-trans-coumaroylquinic acid, norbergenin, arteamisinin I, and gallic acid.

Quercetin has been identified in the leaves of *M. malabathricum*, which was extracted using methanol (Karupiah & Ismail, 2013; Sirat et al., 2010) and ethyl acetate (Susanti et al., 2008). This compound (quercetin) was identified at RT 5.06 with ion precursor m/z 303.0497 [M-H]⁺ (C₁₅H₁₀O₇), which then lost hydroxy molecules at C ring to form C₁₅H₉O₆ (m/z 285.03895) (Figure 5a). Another fragments of quercetin were found at m/z 257.04429 (C₁₄H₉O₅), 229.04912 (C₁₃H₈O₄), 217.04814 (C₁₂H₉O₄), 153.01789 (C₇H₅O₄), and 137.02301 (C₇H₅O₃) (Figure 5b). 4-O-caffeoylquinic acid was found as an antioxidant marker from mulberry leaves extracted with methanol (Ganzon et al., 2018).

Seven other chemical compounds have not been reported in the leaves of *M. malabathricum*. The mass spectrum fragmentation pattern of the sample is to be sure, then compared with previous references or other online databases (PubChem, ChEBI, and HMDB). Ion precursor identified 4-O-caffeoylquinic acid with m/z 355.1024 [M-H]⁺ (C₁₆H₁₈O₉). Another fragmentation pattern of the mass spectrum of 4-O-caffeoylquinic acid compared to the database has a mass error of less than 2.2 mDa, including 337.09156 (C₁₆H₁₇O₈), 245.08033 (C₁₀H₁₃O₇), 193.04972 (C₇H₅O₄), and 149.05946 (C₇H₄O₂) (Figure 5c). 4-O-caffeoylquinic acid was found as an antioxidant marker from mulberry leaves extracted with methanol (Ganzon et al., 2018).

### Table 2. Chemical compounds of the water extract of *M. malabathricum* leaves.

<table>
<thead>
<tr>
<th>Component Name</th>
<th>Chemical Formula</th>
<th>m/z</th>
<th>Neutral mass (Da)</th>
<th>Retention Time (min)</th>
<th>% Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic Acid</td>
<td>C₇H₆O₅</td>
<td>171.0284</td>
<td>170.02152</td>
<td>1.86</td>
<td>0.19</td>
</tr>
<tr>
<td>4-O-Caffeoylquinic Acid</td>
<td>C₁₆H₁₈O₉</td>
<td>355.1024</td>
<td>354.09508</td>
<td>3.57</td>
<td>5.16</td>
</tr>
<tr>
<td>3-O-trans-Coumaroylquinic Acid</td>
<td>C₁₆H₁₈O₈</td>
<td>339.1071</td>
<td>338.10017</td>
<td>4.21</td>
<td>0.43</td>
</tr>
<tr>
<td>Arteamisinine I</td>
<td>C₁₂H₁₈O₂</td>
<td>207.1376</td>
<td>206.13068</td>
<td>4.36</td>
<td>0.38</td>
</tr>
<tr>
<td>Quercimeritrin</td>
<td>C₂₁H₂₀O₁₂</td>
<td>465.1023</td>
<td>464.09548</td>
<td>4.65</td>
<td>1.32</td>
</tr>
<tr>
<td>Quercetin</td>
<td>C₁₅H₁₀O₇</td>
<td>303.0497</td>
<td>302.04265</td>
<td>5.05</td>
<td>24.09</td>
</tr>
<tr>
<td>Norbergenin</td>
<td>C₁₃H₁₄O₉</td>
<td>315.0710</td>
<td>314.06378</td>
<td>5.25</td>
<td>0.42</td>
</tr>
<tr>
<td>Digiprolactone</td>
<td>C₁₁H₁₆O₃</td>
<td>197.1169</td>
<td>196.10994</td>
<td>5.52</td>
<td>0.59</td>
</tr>
</tbody>
</table>

![Figure 4](image-url). LCMS/MS chromatogram profile of water extract of *Melastoma malabathricum* leaves: (a) Quercetin, (b) 4-O-Caffeoylquinic Acid, (c) Quercimeritrin, (d) Digiprolactone, (e) 3-O-trans-Coumaroylquinic Acid, (f) Norbergenin, (g) Arteamisinine I, and (h) Gallic acid. Chromatogram at the upper right corner shows the retention time above 8 minutes by the solvent (methanol).
Phytochemical and antioxidant activity of *Melastoma Malabathricum*

Phytochemical and antioxidant activity of *Melastoma Malabathricum* and rutin) were identified in *Cassia angustifolia* extracted with methanol, ethanol, and ethyl acetate and reported that these extracts have antimicrobial, antioxidant, and anticancer activities (Ahmed et al., 2016).

Furthermore, the ionic precursor identified digiprolactone (Loliolide) with m/z 197.11619 [M-H]+ (C\textsubscript{11}H\textsubscript{16}O\textsubscript{3}), and the fragmentation was then matched against the reported references (Calixto et al., 2017; El Sayed et al., 2020). Also, our study used an online database to ensure these results with mass error < 0.6 mDa of 197.11619, 179.10590, 135.11634, and 105.06922, respectively (Figure 5d). The ionic precursor 3-O-trans-Coumaroylquinic Acid identified, m/z 339.1071 [M-H]+ (C\textsubscript{16}H\textsubscript{18}O\textsubscript{8}), followed by 277.12800 (C\textsubscript{15}H\textsubscript{17}O\textsubscript{5}), 163.03892, 147.04380 (C\textsubscript{6}H\textsubscript{11}O\textsubscript{4}), and 119.04908 (C\textsubscript{8}H\textsubscript{7}O) with mass error < 2.2 mDa from the reported references (Yang et al., 2020), as well as online database (Figure 5e). Digiprolactone, quercetin, and twelve other components were identified in the ethanol extract of *Moringa oleifera* Lam. leaves, and the extract has antibacterial activity (*Staphylococcus aureus*) (Sinaga et al., 2021).

The ionic precursor determined norbergenin with m/z 315.0710 [M-H]+ (C\textsubscript{13}H\textsubscript{14}O\textsubscript{9}) and mass error less than 1.0 mDa (Tenuta et al., 2020; Ukaegbu et al., 2018), and fragmentation followed by 297.06302 (C\textsubscript{13}H\textsubscript{12}O\textsubscript{8}), 153.01782 (C\textsubscript{7}H\textsubscript{6}O\textsubscript{10}), and 125.02266 (C\textsubscript{6}H\textsubscript{5}O\textsubscript{3}) (Figure 5f). Norbergenin was only found in dry leaves of *Arbutus unedo*, which were extracted using the maceration method with ethanol and ethanol in water as solvents (Tenuta et al., 2020) and the methanol extract of the bark of *Diospyros sanza-minika* and has antimalarial activity (Tangmouo et al., 2010). Arteamisinine I was identified at m/z 207.1376 [M-H]+ (C\textsubscript{13}H\textsubscript{18}O\textsubscript{2}) and mass error -0.3 mDa by the ionic precursor (Figure 5g). Arteamisinine I was also found in Huang Hua Hao *Artemisia annua* (Zhou et al., 2011). Another study reporting on this compound is still very limited.

The ionic precursor identified gallic acid with m/z 171.0284 [M-H]+ (C\textsubscript{7}H\textsubscript{6}O\textsubscript{5}) followed by 153.01768 (C\textsubscript{7}H\textsubscript{5}O\textsubscript{4}), 125.02289 (C\textsubscript{6}H\textsubscript{5}O\textsubscript{3}), and 109.02793 (C\textsubscript{6}H\textsubscript{5}O\textsubscript{2}) mass error less than 0.1 mDa (Figure 5h). Previous studies reported gallic acid fragmentation initiated by decarboxylation to pyrogallol (m/z 125.02) and then proceeded to products with low-intensity ions (Syabana et al., 2021). Gallic acid was identified as Grapevine Leaf acetone extract and the extract had antiradical activity against DPPH (Amarowicz et al., 2010).

These compounds were previously reported to have DPPH antioxidant activity; gallic acid (Takao et al., 2015), 4-O-cafeoylquinic acid (Herawati et al., 2019; Zhou et al., 2020), quercimeritrin (Bazylko et al., 2012), quercetin (Takao et al., 2015; Zahratunnisa et al., 2017), norbergenin (Takahashi et al., 2003), and digiprolactone (Zhao et al., 2011). Quercetin and gallic acid are compounds that have been widely reported to have antioxidant activity (DPPH). Quercetin has a higher antioxidant activity than gallic acid (Limanto et al., 2019). Previous research found that quercetin from ethyl acetate extract of *M. malabathricum* leaves was the most active free radical scavenger by the DPPH method (IC\textsubscript{50} 0.21 ppm). Their structure is responsible for the antioxidant capacity of phenolic consist of one (phenolic acid) or more (polyphenol) aromatic rings with hydroxyl groups. Furthermore, the number and position of the hydroxyl group and the type of substitution on the aromatic ring are responsible for neutralizing free radicals. The hydrogen atoms of the adjacent hydroxyl group (o-diphenol) at various positions (A, B, and C rings), as well as the double bond of the benzene ring and the oxo functional group (-C = O), provide high antioxidant activity.

Figure 5. MS\textsuperscript{2} spectrum and mass fragmentations (a) Quercetin, (b) 4-O-Caffeoylquinic Acid, (c) Quercimeritrin, (d) Digiprolactone, (e) 3-O-trans-Coumaroylquinic Acid, (f) Norbergenin, (g) Arteamisinine I, and (h) Gallic acid.
(Minatel et al., 2017). The chemical structure of quercetin has a higher number of hydrogen atoms from the hydroxyl group and has double bonds at 2,3 and 4-oxo than gallic acid. Therefore, it might have elevated antioxidant capacity.

The IC₅₀ value of the water extract of *M. malabathricum* leaves (IC₅₀ 3.04 ± 0.31 ppm) is smaller than vitamin C (IC₅₀ 4.32 ± 0.16 ppm). Thus, *M. malabathricum* leaves may have the potential to be developed as a functional food. Further research in vivo and in silico is needed to confirm this result. An antioxidant compound in vitro did not employ a similar way with in vivo system. It is altered by the structural chemistry of both reagents and reaction conditions (Santos-Sánchez et al., 2019).

4 Conclusions

The extraction condition of *M. malabathricum* leaves of 5 g/100 mL with hot water (90 ± 2 °C) for 15 minutes revealed the highest antioxidant activity. Water extract of *M. malabathricum* leaves has a potential role as an antioxidant (IC₅₀ 3.09 ± 0.21 ppm) and low toxicity (IC₅₀ 381.80 ± 94.36 ppm). Gallic acid, 4-O-caffeoylquinic acid, 3-O-trans-caftaroylquinic acid, artequinisin I, quercirin, quercetin, norberginin, and digirolactone were contained in the water extract of *M. malabathricum* leaves. Further research in vivo is needed to determine the antioxidant potential of the water extract of *M. malabathricum* leaves intensely.

Acknowledgements

This research was funded by the Ministry of Research and Technology/National Research and Innovation Agency of Republic Indonesia.

References


