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

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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 °C and 90 ± 2 °C), 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 °C) 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 arteamisinin 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 °C) 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-Mossoń & 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 muricate* (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.

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The methanolic extract of the *M. malabathricum* leaves contained several bioactive compounds. These compounds were asiatic acid, ellagic acid, b-sitosterol 3-O-b-D-glucopyranoside, 2a-hydroxyursolic acid, glycolipid glycerol, 1,2-dilinolenyl-3-O-b-D-galactopyanoside, 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, patriscabratine, quercitrin, kaempferol-3-O-(2", 6"-di-O-p-trans-coumaroyl)-β-glucoside, and quercetin (Sirat et al., 2010). The study on the phytochemical profil 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 profil 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 IC_{50} 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 µL 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 LC_{50} value. Measurements were triplicate. The LC_{50} 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

Table 1. Nine extraction conditions of *M. malabathricum* dried powdered leaves.

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

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.

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 °C. 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 °C, 10 g/100 mL, 45 min) and H1030 (90 ± 2 °C, 10 g/100 mL, 30 min), had the highest extraction yield followed by H0545 (90 ± 2 °C, 5 g/100 mL, 45 min), and H0530 (90 ± 2 °C, 5 g/100 mL, 30 min). In contrast, N1015 had the lowest yield (25 ± 2 °C, 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 °C for 20 minutes (Balci & Özdemir, 2016).

Nine extraction conditions of *M. malabathricum* leaves generated significantly different antioxidant activity with IC_{50} of 2.13 – 19.20 ppm ($p < 0.05$). The lowest antioxidant activity is shown in H1045 (90 ± 2 °C, 10 g/100 mL, 45 minutes) followed by N1045 (25 ± 2 °C, 10 g/100 mL, 45 minutes). The other seven extraction conditions had the lowest values, which were not significantly different ($P > 0.05$).

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.

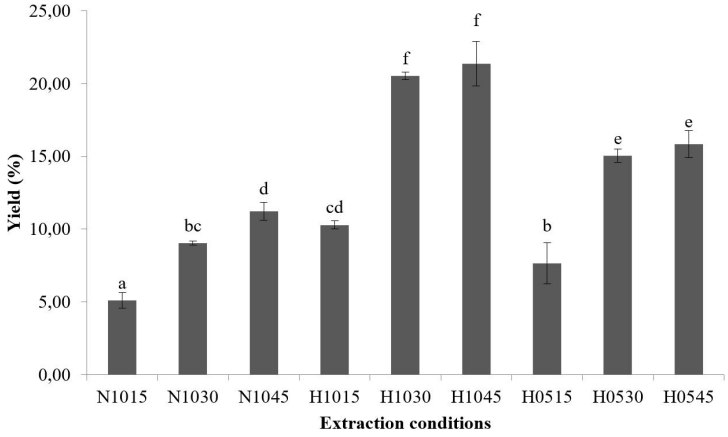

Figure 1. Extract yield obtained by several extraction conditions. The different letter show the result are significantly different ($p < 0.05$). Sample code explanation is provided Table 1. The first letter of the code indicates the temperature of the water used is normal (N = 25 ± 2 °C) or hot (H = 90 ± 2 °C), the first two numbers indicate the concentration of leaves (g/mL), and the next two numbers indicate the extraction time (min).

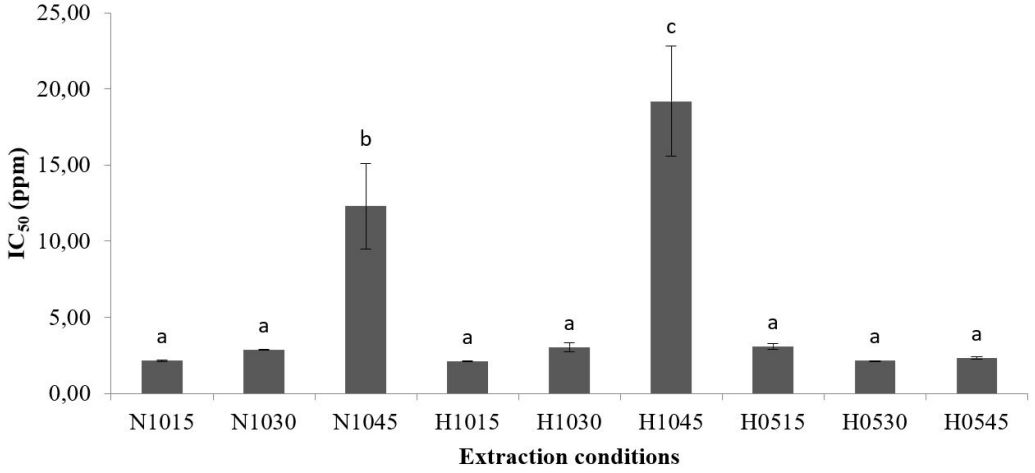

Figure 2. Antioxidant activity obtained by several extraction conditions. The different letter show the result are significantly different ($p < 0.05$). Sample code explanation is provided Table 1. The first letter of the code indicates the temperature of the water used is normal (N = 25 ± 2 °C) or hot (H = 90 ± 2 °C), the first two numbers indicate the concentration of leaves (g/mL), and the next two numbers indicate the extraction time (min).

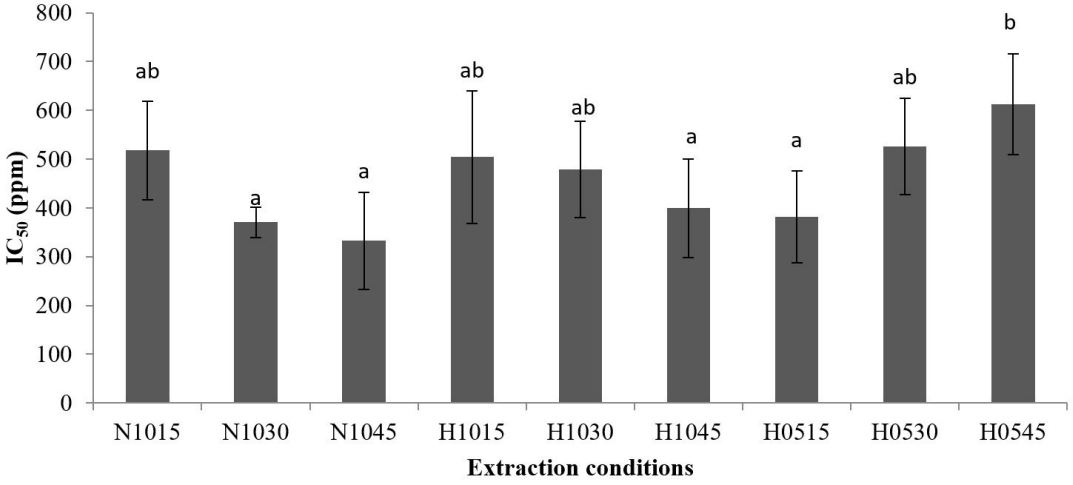

Figure 3. Toxicity obtained by several extraction conditions. The different letter show the result are significantly different ($p < 0.05$). Sample code explanation is provided Table 1. The first letter of the code indicates the temperature of the water used is normal (N = 25 ± 2 °C) or hot (H = 90 ± 2 °C), the first two numbers indicate the concentration of leaves (g/mL), and the next two numbers indicate the extraction time (min).

Nine extraction conditions of *M. malabathricum* leaves generated significantly different toxicity with an IC_{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_{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 (IC50 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 chracaterized using LC-MS. Based on the Unifi system and Chemspider 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 $[M-H]^+$ ($C_{15}H_{10}O_7$), which then lost hydroxy molecules at C ring to form $C_{15}H_9O_6$ (m/z 285.03895) (Figure 5a). Another fragments of quercetin were found at m/z 257.04429 ($C_{14}H_9O_5$), 229.04912 ($C_{13}H_9O_4$), 217.04814 ($C_{12}H_9O_4$), 153.01789 ($C_7H_5O_4$), and 137.02301 ($C_7H_5O_3$). The fragmentations patterns of the quercetin mass spectra compared with the reported references (Li et al., 2016; Scigelova et al., 2011) and the Human Metabolome (HMDB) database has a mass error of less than 0.2 mDa. Quercetin was identified in the ethanol extract of the leaves of Gandaria (*Bouea Macrophylla* Griff.) and the extract had a higher antioxidant activity than the extract with hexane and ethyl acetate (Hardinsyah et al., 2019).

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 $[M-H]^+$ ($C_{16}H_{18}O_9$). 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 ($C_{16}H_{17}O_8$), 245.08033 ($C_{10}H_{13}O_7$), 193.04972 ($C_7H_{13}O_6$), and 149.05946 ($C_6H_{13}O_4$) (Figure 5b). 4-O-caffeoylquinic acid was found as an antioxidant marker from mulberry leaves extracted with methanol (Ganzon et al., 2018).

The ionic precursor identified quercimeritrin with m/z 465.1023 $[M-H]^+$ ($C_{21}H_{20}O_{12}$), and the fragmentation was then matched against the database (mass error < 0.3 mDa), 303.0497 ($C_{15}H_{10}O_7$), 285.03833 ($C_{15}H_8O_6$), and 137.02288 ($C_7H_6O_3$) (Figure 5c). Quercimeritrin and two other compounds (scutellarein

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

Component Name	Chemical Formula	m/z	Neutral mass (Da)	Retention Time (min)	% Area (%)
Gallic Acid	$C_7H_6O_5$	171.0284	170.02152	1.86	0.19
4-O-Caffeoylquinic Acid	$C_{16}H_{18}O_9$	355.1024	354.09508	3.57	5.16
3-O-trans-Coumaroylquinic Acid	$C_{16}H_{18}O_8$	339.1071	338.10017	4.21	0.43
Arteamisinine I	$C_{13}H_{18}O_2$	207.1376	206.13068	4.36	0.38
Quercimeritrin	$C_{21}H_{20}O_{12}$	465.1023	464.09548	4.65	1.32
Quercetin	$C_{15}H_{10}O_7$	303.0497	302.04265	5.05	24.09
Norbergenin	$C_{13}H_{14}O_9$	315.0710	314.06378	5.25	0.42
Digiprolactone	$C_{11}H_{16}O_3$	197.1169	196.10994	5.52	0.59

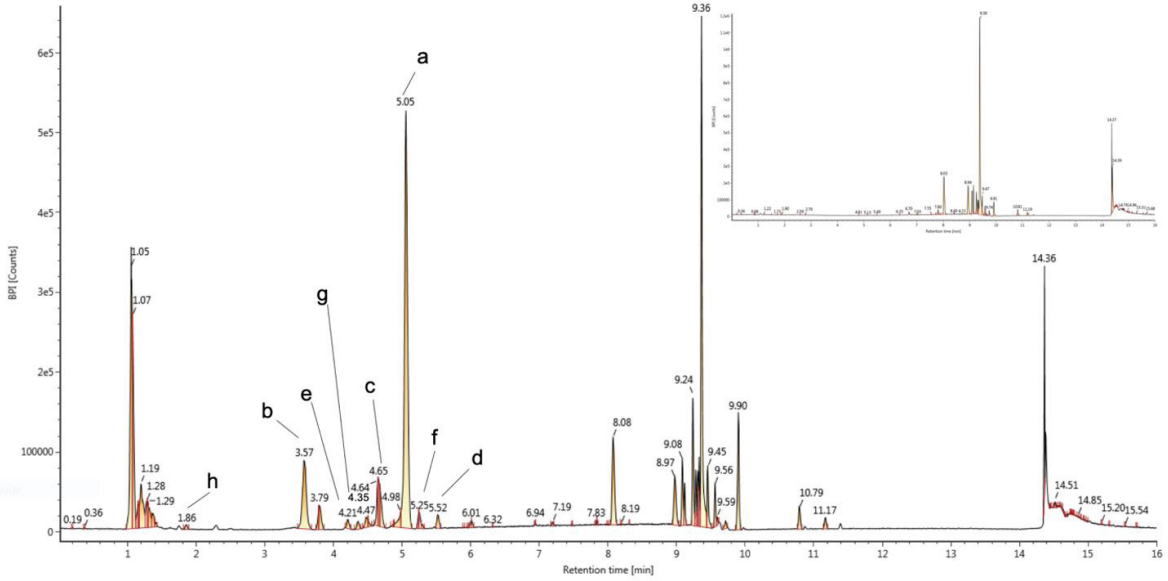

Figure 4. LCMS/MS chromatogram profile of water extract of *Melastoma malabathricum* leaves: (a) Quercetin, (b) 4-O-Caffeoylquinic Acid, (c) Quercimeritrin, (d) Digiprolactone, (e) 3-O-trans-Coumaroylquinic Acid, (f) Norbergenin, (g) Arteamisinine I, and (h) Gallic acid. Chromatogram at the upper right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

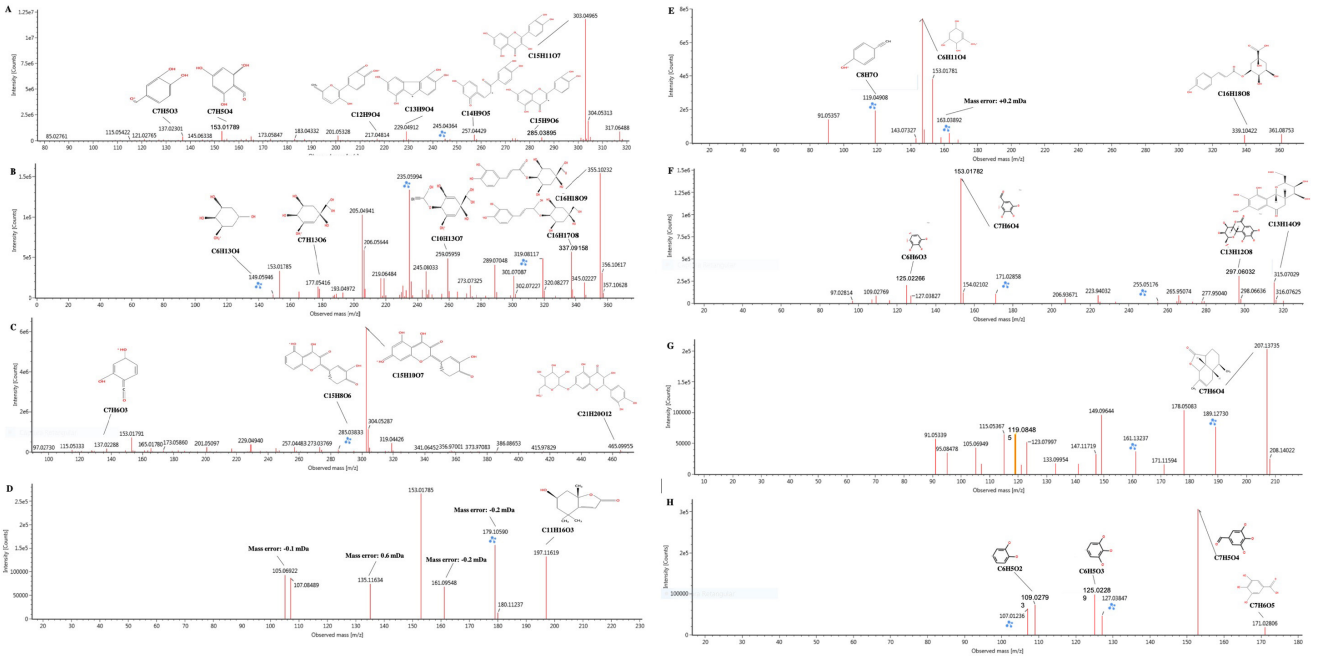

Figure 5. MS² spectrum and mass fragmentations (a) Quercetin, (b) 4-O-Caffeoylquinic Acid, (c) Quercimeritrin, (d) Digiprolactone, (e) 3-O-trans-Coumaroylquinic Acid, (f) Norbergenin, (g) Arteamisinine I, and (h) Gallic acid.

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 $[M-H]^+$ ($C_{11}H_{16}O_3$), 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, 161.09548, 135.11634, and 105.06922, respectively (Figure 5d). The ionic precursor 3-O-trans-Coumaroylquinic Acid identified, m/z 339.1071 $[M-H]^+$ ($C_{16}H_{18}O_8$), followed by 277.12800 ($C_{15}H_{17}O_5$), 163.03892, 147.04380 ($C_6H_{11}O_4$), and 119.04908 (C_8H_7O) 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]+ ($C_{13}H_{14}O_9$) and mass error less than 1.0 mDa (Tenuta et al., 2020; Ukaegbu et al., 2018), and fragmentation followed by 297.06032 ($C_{13}H_{12}O_8$), 153.01782 ($C_7H_6O_{10}$), and 125.02266 ($C_6H_6O_3$) (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 $[M-H]^+$ ($C_{13}H_{18}O_2$) 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]⁺ (C7H6O5) followed by 153.01768 ($C_7H_5O_4$), 125.02289 ($C_6H_5O_3$), and 109.02793 ($C_6H_5O_2$) 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-caffeoylquinic acid (Herawati et al., 2019; Zhou et al., 2020), quercimeritrin (Bazylko et al., 2012), quercetin (Takao et al., 2015; Zahratunnisa et al., 2017), norberginin (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_{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

(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 IC_{50} value of the water extract of *M. malabathricum* leaves (IC_{50} 3.04 ± 0.31 ppm) is smaller than vitamin C (IC_{50} 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 (IC_{50} 3.09 ± 0.21 ppm) and low toxicity (LC_{50} 381.80 ± 94.36 ppm). Gallic acid, 4-O-caffeoylquinic acid, 3-O-trans-coumaroylqinic acid, arteamisinin I, quercimeritrin, quercetin, norberginin, and digiprolactone 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.

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References

Ahmed, S. I., Hayat, M. Q., Tahir, M., Mansoor, Q., Ismail, M., Keck, K., & Bates, R. B. (2016). Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of Cassia angustifolia Vahl. *BMC Complementary and Alternative Medicine*, 16(1), 460. http://dx.doi.org/10.1186/s12906-016-1443-z. PMid:27835979.

Alizadeh, A. H. M. (2020). Diabetes mellitus and pancreas: an overview. *Journal of the Pancreas*, 21(4), 72-73.

Amarowicz, R., Weidner, S., Wójtowicz, I., Karamać, M., Kosińska, A., & Rybarczyk, A. (2010). Influence of low-temperature stress on changes in the composition of grapevine leaf phenolic compounds and their antioxidant properties. *Functional Plant Science & Biotechnology*, 4, 90-96.

Balamurugan, K., Nishanthini, A., & Mohan, V. R. (2014). Antidiabetic and antihyperlipidaemic activity of ethanol extract of Melastoma malabathricum Linn. leaf in alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine*, 4(Suppl. 1), S442-S448. http://dx.doi.org/10.12980/APJTB.4.2014C122. PMid:25183126.

Balci, F., & Özdemir, F. (2016). Influence of shooting period and extraction conditions on bioactive compounds in Turkish green tea. *Food Science and Technology*, 36(4), 737-743. http://dx.doi.org/10.1590/1678-457x.17016.

Bazylko, A., Stolarczyk, M., Derwińska, M., & Kiss, A. K. (2012). Determination of antioxidant activity of extracts and fractions obtained from Galinsoga parviflora and Galinsoga quadriradiata, and a qualitative study of the most active fractions using TLC and HPLC methods. *Natural Product Research*, 26(17), 1584-1593. http://dx.doi.org/10.1080/14786419.2011.582469. PMid:22085305.

Calixto, N. O., Cordeiro, M. S., Giorno, T. B. S., Oliveira, G. G., Lopes, N. P., Fernandes, P. D., Pinto, A. C., & Rezende, C. M. (2017). Chemical constituents of Psychotria nemorosa gardner and antinociceptive activity. *Journal of the Brazilian Chemical Society*, 28(5), 707-723.

Campbell, C. G., Picotte, M. S., Syndergaard, S., Filipowicz, R., & Thorland, W. G. (2010). Anti-inflammatory effects of camelina sativa oil in postmenopausal women. *The FASEB Journal*, 23, 910.14.

Chang, M. Y., Lin, Y. Y., Chang, Y. C., Huang, W. Y., Lin, W. S., Chen, C. Y., Huang, S. L., & Lin, Y. S. (2020). Effects of infusion and storage on antioxidant activity and total phenolic content of black tea. *Applied Sciences*, 10(8), 2685. http://dx.doi.org/10.3390/app10082685.

Chauhan, P., Pandey, I., & Kumar Dhatwalia, V. (2010). Evaluation of the anti-diabetic effect of ethanolic and methanolic extracts of centella asiatica leaves extract on alloxan induced diabetic rats. *Advances in Biological Research*, 4(1), 27-30.

D'Oria, R., Schipani, R., Leonardini, A., Natalicchio, A., Perrini, S., Cignarelli, A., Laviola, L., & Giorgino, F. (2020). The role of oxidative stress in cardiac disease: from physiological response to injury factor. *Oxidative Medicine and Cellular Longevity*, 2020, 5732956. http://dx.doi.org/10.1155/2020/5732956. PMid:32509147.

El Sayed, A. M., Basam, S. M., El-Naggar, E. M., Marzouk, H. S., & El-Hawary, S. (2020). LC–MS/MS and GC–MS profiling as well as the antimicrobial effect of leaves of selected Yucca species introduced to Egypt. *Scientific Reports*, 10(1), 17778. http://dx.doi.org/10.1038/s41598-020-74440-y. PMid:33082381.

Faraone, I., Rai, D. K., Russo, D., Chiummiento, L., Fernandez, E., Choudhary, A., & Milella, L. (2019). Antioxidant, antidiabetic, and anticholinesterase activities and phytochemical profile of Azorella glabra wedd. *Plants*, 8(8), 1-14. http://dx.doi.org/10.3390/plants8080265. PMid:31382601.

Ganzon, J. G., Chen, L. G., & Wang, C. C. (2018). 4-O-Caffeoylquinic acid as an antioxidant marker for mulberry leaves rich in phenolic compounds. *Journal of Food and Drug Analysis*, 26(3), 985-993. PMid:29976416.

Gheith, I., & El-Mahmoudy, A. (2018). Laboratory evidence for the hematopoietic potential of beta vulgaris leaf and stalk extract in a phenylhydrazine model of anemia. *Brazilian Journal of Medical and Biological Research*, 51(11), e7722. http://dx.doi.org/10.1590/1414-431x20187722. PMid:30328935.

Hardinsyah, H., Windardi, I. P., Aries, M., & Damayanthi, E. (2019). Total phenolic content, quercetin, and antioxidant activity of gandaria (Bouea Macrophylla Griff.) leaf extract at two stages of maturity. *Jurnal Gizi Dan Pangan*, 14(2), 61-68. http://dx.doi.org/10.25182/jgp.2019.14.2.61-68.

Herawati, D., Giriwono, P. E., Dewi, F. N. A., Kashiwagi, T., & Andarwulan, N. (2019). Three major compounds showing significant antioxidative, α-glucosidase inhibition, and antiglycation activities in Robusta coffee brew. *International Journal of Food Properties*, 22(1), 994-1010. http://dx.doi.org/10.1080/10942912.2019.1622562.

Hosseini, R., Shokoohi-Nahrkhalaji, A., ZeinaliNia, E., Samimi-Motlagh, F., Ahmadi, R., & Shahmoradi, H. (2014). Free radical capacity in plasma of type 2 diabetic patients. *Journal of Biology and Today's World*, 3(7), 162-165. http://dx.doi.org/10.15412/J.JBTW.01030705.

Hou, W., Zhang, W., Chen, G., & Luo, Y. (2016). Optimization of extraction conditions for maximal phenolic, flavonoid and antioxidant activity from melaleuca bracteata leaves using the response surface methodology. *PLoS One*, 11(9), e0162139. http://dx.doi.org/10.1371/journal.pone.0162139. PMid:27611576.

Karamać, M., Gai, F., & Peiretti, P. G. (2020). Eff ect of the growth stage of false flax (camelina sativa l.) on the phenolic compound content and antioxidant potential of the aerial part of the plant. *Polish Journal of Food and Nutrition Sciences*, 70(2), 189-198. http://dx.doi.org/10.31883/pjfns/119719.

Karupiah, S., & Ismail, Z. (2013). Antioxidative effect of melastoma malabathticum l extract and determination of its bioactive flavonoids from various location in malaysia by RP-HPLC with diode array detection. *Journal of Applied Pharmaceutical Science*, 3(2), 19-24.

Kasunmala, I. G. G., Navaratne, S. B., & Wickramasinghe, I. (2020). Antioxidant activity and physicochemical properties changes of Melastoma malabathricum (L.) and Syzygium Caryophyllatum (L.) fruit during ripening. *International Journal of Fruit Science*, 20(S3), S1819-S1828. http://dx.doi.org/10.1080/15538362.2020.1834896.

Kawamura, T., & Muraoka, I. (2018). Exercise-induced oxidative stress and the effects of antioxidant intake from a physiological viewpoint. *Antioxidants*, 7(9), 119. http://dx.doi.org/10.3390/antiox7090119. PMid:30189660.

Kumar, V., Ahmed, D., Gupta, P. S., Anwar, F., & Mujeeb, M. (2013). Anti-diabetic, anti-oxidant and anti-hyperlipidemic activities of Melastoma malabathricum Linn. leaves in streptozotocin induced diabetic rats. *BMC Complementary and Alternative Medicine*, 13(1), 222. http://dx.doi.org/10.1186/1472-6882-13-222. PMid:24010894.

Lemus-Mondaca, R., Vega-Gálvez, A., Rojas, P., Stucken, K., Delporte, C., Valenzuela-Barra, G., Jagus, R. J., Agüero, M. V., & Pasten, A. (2018). Antioxidant, antimicrobial and anti-inflammatory potential of Stevia rebaudiana leaves: effect of different drying methods. *Journal of Applied Research on Medicinal and Aromatic Plants*, 11, 37-46. http://dx.doi.org/10.1016/j.jarmap.2018.10.003.

Li, Z. H., Guo, H., Xu, W., Ge, J., Li, X., Alimu, M., & He, D. J. (2016). Rapid identification of flavonoid constituents directly from PTP1B inhibitive extract of raspberry (Rubus idaeus L.) leaves by HPLC-ESI-QTOF-MS-MS. *Journal of Chromatographic Science*, 54(5), 805-810. http://dx.doi.org/10.1093/chromsci/bmw016. PMid:26896347.

Limanto, A., Simamora, A., Santoso, A. W., & Timotius, K. H. (2019). Antioxidant, α-Glucosidase inhibitory activity and molecular docking study of gallic acid, quercetin and rutin: a comparative study. *Molecular and Cellular Biomedical Sciences*, 3(2), 67-74. http://dx.doi.org/10.21705/mcbs.v3i2.60.

Marliani, L., Budiana, W., & Anandari, Y. (2017). The effect of extraction condition on the polyphenol content and antioxidant activity of Curcuma zedoaria (Christm.). *Ijpst*, 4(2), 57-63. http://dx.doi.org/10.15416/ijpst.v4i2.12770.

Marzuki, A., Rahman, L., & Mamada, S. S. (2019). Toxicity test of stem bark extract of banyuru (Pterospermum celebicum miq.) using BSLT (brine shrimp lethality test) and cream irritation test. *Journal of Physics: Conference Series*, 1341(7), 072018. http://dx.doi.org/10.1088/1742-6596/1341/7/072018.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, 45(1), 31-34. http://dx.doi.org/10.1055/s-2007-971236. PMid:7100305.

Minatel, I. O., Borges, C. V., Ferreira, M. I., Gomez, H. A. G., Chen, C.-Y. O., & Lima, G. P. P. (2017). Phenolic compounds: functional properties, impact of processing and bioavailability. In M. Soto-Hernandez, M. Palma-Tenango & M. R. Garcia-Mateos (Eds.), *Phenolic compounds biological activity* (pp. 1-24). London: IntechOpen. http://dx.doi.org/10.5772/66368.

Młynarczyk, K., & Walkowiak-Tomczak, D. (2017). Bioactive properties of elderflowers (Sambucus nigra L.). *World Science News*, 73(732), 115-119.

Nikniaz, Z., Mahdavi, R., Ghaemmaghami, S. J., Lotfi Yagin, N., & Nikniaz, L. (2016). Effect of different brewing times on antioxidant activity and polyphenol content of loosely packed and bagged black teas (Camellia sinensis L.). *Avicenna Journal of Phytomedicine*, 6(3), 313-321. PMid:27462554.

Orak, H. H., Bahrisefit, I. S., & Sabudak, T. (2019). Antioxidant activity of extracts of soursop (Annona muricata L.) leaves, fruit pulps, peels, and seeds. *Polish Journal of Food and Nutrition Sciences*, 69(4), 359-366. http://dx.doi.org/10.31883/pjfns/112654.

Pashkow, F. J. (2011). Oxidative stress and inflammation in heart disease: do antioxidants have a role in treatment and/or prevention? *International Journal of Inflammation*, 2011(4), 514623. http://dx.doi.org/10.4061/2011/514623. PMid:21860805.

Rafi, M., Meitary, N., Septaningsih, D. A., & Bintang, M. (2020). Phytochemical profile and antioxidant activity of guazuma ulmifolia leaves extracts using different solvent extraction. *Indonesian Journal of Pharmacy*, 31(3), 171-180. http://dx.doi.org/10.22146/ijp.598.

Rafi, M., Rismayani, W., Sugiarti, R. M., Syafitri, U. D., Wahyuni, W. T., & Rohaeti, E. (2021). FTIR-based fingerprinting combined with chemometrics for discrimination of Sonchus arvensis leaves extracts of various extracting solvents and the correlation with its antioxidant activity. *Indonesian Journal of Pharmacy*, 32(2), 132-140. http://dx.doi.org/10.22146/ijp.755.

Reuter, S., Gupta, S. C., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: how are they linked? *Free Radical Biology & Medicine*, 49(11), 1603-1616. http://dx.doi.org/10.1016/j.freeradbiomed.2010.09.006. PMid:20840865.

Robles, L., Vaziri, N. D., & Ichii, H. (2013). Role of oxidative stress in the pathogenesis of pancreatitis: effect of antioxidant therapy. *Pancreatic Disorders & Therapy*, 3(1), 112. http://dx.doi.org/10.4172/2165-7092.1000112. PMid:24808987.

Santos-Sánchez, N. F., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019). Antioxidant compounds and their antioxidant mechanism. *Antioxidants*, 1-28.

Scigelova, M., Hornshaw, M., Giannakopulos, A., & Makarov, A. (2011). Fourier transform mass spectrometry. *Molecular & Cellular Proteomics*, 10(7), 009431. http://dx.doi.org/10.1074/mcp.M111.009431. PMid:21742802.

Senoner, T., & Dichtl, W. (2019). Oxidative stress in cardiovascular diseases: still a therapeutic target? *Nutrients*, 11(9), 2090. http://dx.doi.org/10.3390/nu11092090. PMid:31487802.

Sinaga, N. I., Hanafi, M., & Yantih, N. (2021). Identification of chemical compounds and antibacterial activity of 96% ethanol extract from moringa oleifera lam. Leaves against mrsa (methicillin resistant staphylococcus aureus). *International Journal of Applied Pharmaceutics*, 13(2), 111-114. http://dx.doi.org/10.22159/ijap.2021.v13s2.21.

Sirat, H. M., Susanti, D., Ahmad, F., Takayama, H., & Kitajima, M. (2010). Amides, triterpene and flavonoids from the leaves of Melastoma malabathricum L. *Journal of Natural Medicines*, 64(4), 492-495. http://dx.doi.org/10.1007/s11418-010-0431-8. PMid:20582481.

Śliwińska-Mossoń, M., & Milnerowicz, H. (2017). The impact of smoking on the development of diabetes and its complications. *Diabetes and Vascular Disease Research*, 14(4), 265-276. http://dx.doi.org/10.1177/1479164117701876. PMid:28393534.

Susanti, D., Sirat, H., Ahmad, F., & Ali, R. (2008). Bioactive constituents from the leaves of Melastoma malabathricum L. *Jurnal Ilmiah Farmasi UII*, 5(1), 96438.

Syabana, M. A., Yuliana, N. D., Batubara, I., Fardiaz, D., Sciences, N., & Biopharmaca, T. (2021). Characterization of antioxidant compound

from Syzygium polyanthum leaves extract using UHPLC-HRM. *Molekul*, 16(1), 38-45. http://dx.doi.org/10.20884/1.jm.2021.16.1.666.

Takahashi, H., Kosaka, M., Watanabe, Y., Nakade, K., & Fukuyama, Y. (2003). Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity. *Bioorganic & Medicinal Chemistry*, 11(8), 1781-1788. http://dx.doi.org/10.1016/S0968-0896(02)00666-1. PMid:12659764.

Takao, L. K., Imatomi, M., & Gualtieri, S. C. J. (2015). Antioxidant activity and phenolic content of leaf infusions of Myrtaceae species from Cerrado (Brazilian Savanna). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 75(4), 948-952. http://dx.doi.org/10.1590/1519-6984.03314. PMid:26675912.

Tangmouo, J. G., Ho, R., Matheeussen, A., Lannang, A. M., Komguem, J., Messi, B. B., Maes, L., & Hostettmann, K. (2010). Antimalarial activity of extract and norbergenin derivatives from the stem bark of Diospyros sanza-minika A. Chevalier (Ebenaceae). *Phytotherapy Research*, 24(11), 1676-1679. http://dx.doi.org/10.1002/ptr.3192. PMid:21031627.

Tenuta, M. C., Deguin, B., Loizzo, M. R., Dugay, A., Acquaviva, R., Malfa, G. A., Bonesi, M., Bouzidi, C., & Tundis, R. (2020). Contribution of flavonoids and iridoids to the hypoglycaemic, antioxidant, and nitric oxide (NO) inhibitory activities of arbutus unedo L. *Antioxidants*, 9(2), 1-25. http://dx.doi.org/10.3390/antiox9020184. PMid:32098404.

Tsykalo, T. O., & Trzhetsynskyi, S. D. (2020). The study of hypoglycemic and hypolipidemic activity of Camelina sativa (L.) Crantz extracts in rats under conditions of high-fructose diet. *Ceska a Slovenska Farmacie*, 69(3), 137-142. PMid:32972157.

Ukaegbu, C. I., Shah, S. R., Hazrulrizawati, A. H., & Alara, O. R. (2018). Acetone extract of Flammulina velutipes caps: a promising source of antioxidant and anticancer agents. *Beni-Suef University Journal of Basic and Applied Sciences*, 7(4), 675-682. http://dx.doi.org/10.1016/j.bjbas.2018.07.012.

Vergara-Salinas, J. R., Pérez-Jiménez, J., Torres, J. L., Agosin, E., & Pérez-Correa, J. R. (2012). Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (Thymus vulgaris). *Journal of Agricultural and Food Chemistry*, 60(44), 10920-10929. http://dx.doi.org/10.1021/jf3027759. PMid:23075096.

Wong, K. C., Hag Ali, D. M., & Boey, P. L. (2012). Chemical constituents and antibacterial activity of Melastoma malabathricum L. *Natural Product Research*, 26(7), 609-618. http://dx.doi.org/10.1080/14786419.2010.538395. PMid:21834640.

Yang, L., Fang, Y., Liu, R., & He, J. (2020). Phytochemical analysis, anti-inflammatory, and antioxidant activities of dendropanax dentiger roots. *BioMed Research International*, 2020, 5084057. http://dx.doi.org/10.1155/2020/5084057. PMid:33294445.

Zahratunnisa, N., Elya, B., & Noviani, A. (2017). Inhibition of Alpha-Glucosidase and antioxidant test of stem bark extracts of garcinia fruticosa lauterb. *Pharmacognosy Journal*, 9(2), 273-275. http://dx.doi.org/10.5530/pj.2017.2.46.

Zakaria, Z. A., Balan, T., Mamat, S. S., Mohtarrudin, N., Kek, T. L., & Salleh, M. Z. (2015). Mechanisms of gastroprotection of methanol extract of Melastoma malabathricum leaves. *BMC Complementary and Alternative Medicine*, 15(1), 135. http://dx.doi.org/10.1186/s12906-015-0638-z. PMid:25927982.

Zakaria, Z. A., Jaios, E. S., Omar, M. H., Abd. Rahman, S., Hamid, S. S. A., Ching, S. M., Teh, L. K., Salleh, M. Z., Deny, S., & Taher, M. (2016). Antinociception of petroleum ether fraction derived from crude methanol extract of Melastoma malabathricum leaves and its possible mechanisms of action in animal models. *BMC Complementary and Alternative Medicine*, 16(1), 488. http://dx.doi.org/10.1186/s12906-016-1478-1. PMid:27899097.

Zhao, J., Zhou, L., Li, X., Xiao, H., Hou, F., & Cheng, Y. (2011). Bioactive compounds from the aerial parts of Brachystemma calycinum and structural revision of an Octacyclopeptide. *Journal of Natural Products*, 74(6), 1392-1400. http://dx.doi.org/10.1021/np200048u. PMid:21634415.

Zheng, W. J., Ren, Y. S., Wu, M. L., Yang, Y. L., Fan, Y., Piao, X. H., Ge, Y. W., & Wang, S. M. (2021). A review of the traditional uses, phytochemistry and biological activities of the Melastoma genus. *Journal of Ethnopharmacology*, 264, 113322. http://dx.doi.org/10.1016/j.jep.2020.113322. PMid:32871236.

Zhou, J., Xie, G., & Yan, X. (2011). *Encyclopedia of traditional Chinese Medicines: molecular structures, pharmacological activities, natural sources and applications* (Vol. 1). Heidelberg: Springer.

Zhou, R. R., Liu, X. H., Chen, L., Huang, J. H., Liang, X. J., Wan, D., Zhang, S. H., & Huang, L. Q. (2020). Comparison of the antioxidant activities and phenolic content of five lonicera flowers by HPLC-DAD/MS-DPPH and chemometrics. *International Journal of Analytical Chemistry*, 2020, 2348903. http://dx.doi.org/10.1155/2020/2348903. PMid:32308684.