Quantitative analysis study on marker compound in crude extract and polar fraction of *Melastoma malabathricum* L. with reversed-phase high-performance liquid chromatography and determination of antioxidant activity and sun protection factor value as natural resources for cosmetics

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**ABSTRACT**
Cosmetics are all the rage these days and are considered essential products. With the high consumption of beauty and body care products, it is a great challenge to produce cosmetic ingredients from nature. This study identifies chemical compounds with high phenol content by separating compounds with different solubility and testing levels of secondary metabolite markers. The sample in this study is a soluble compound in polar solvents, namely crude extract, ethyl acetate fraction, ethanol, and residue fraction. Determination begins in this *Melastoma malabathricum* L. study starting with thin-layer chromatography (TLC) as qualitative analysis and quantitative analysis of compound content using reversed-phase high-performance liquid chromatography (RP-HPLC) with gallic acid and quercetin as marker compounds, antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and ferric reduction antioxidant power (FRAP) methods, and sun protection factor (SPF) values for each sample. The TLC test’s retention factor values for each sample, test result: crude extract, ethyl acetate, and ethanol fraction of 0.15 for gallic acid and 0.38 for quercetin. In a quantitative test using RP-HPLC mobile phase phosphoric acid 0.05% pH 3.42-acetonitrile (60:40) for gallic acid and phosphate buffer pH 5.4-acetonitrile (60:40) for quercetin. The result showed that gallic acid levels in crude extract, ethyl acetate, ethanol fraction, and residue are 10.25 ± 0.59 mg/g, 49.29 ± 0.49 mg/g, 6.43 ± 0.14 mg/g, and 7.54 ± 0.61 mg/g, respectively, and quercetin content in crude extract, ethyl acetate, ethanol fraction, and residue was 0.40 ± 2.03 mg/g, 0.93 ± 0.06 mg/g, 0.86 ± 2.99 mg/g, and 0.03 ± 3.74 mg/g, respectively. The result of antioxidant activity from the DPPH and FRAP assay showed that the ethyl acetate fraction has the highest activity compared to vitamin C and quercetin standard and IC₅₀ of ethyl acetate is 1.9 ± 0.12 and 4.2 ± 1.5 µg/ml, respectively. Identification of SPF result ethyl acetate fraction with the highest value is 59.3 ± 0.9. The result showed that ethyl acetate fraction has the potential to be a raw material for the formulation of sunscreen preparations with SPF values included in the ultracategory.

**INTRODUCTION**
Skin is the primary layer of living things that functions like a coating for organs. Excessive exposure to ultraviolet radiation can cause skin damage. Damage that occurs can be immediate or long-term (*Kumar et al.*, 2019). The report provided by the Global Cancer Observatory Platform was known in 2019.
More than 287,723 cases of malignant melanoma occurred due to ultraviolet (UV) radiation with a mortality ratio of 21% (WHO, 2019). The skin’s epidermis protects the inner skin from potentially hazardous environments such as chemicals and UV radiation (Kim et al., 2004). It is known that the wavelength of UV light consists of UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm). UVB can penetrate the epidermis layer of the skin, stimulating excess reactive oxygen species (ROS) production in the skin (Evans and Johnson, 2010; Hwang et al., 2013a, 2013b). ROS causes a significant problem in the photoaging process. The mechanism is inducing matrix metalloproteinases, which break down the extracellular matrix by inhibiting the expression of procollagen (Mani et al., 2013; Park et al., 2012).

Photooxidative damage can affect cellular lipids, proteins, and DNA. Photooxidative damage causes erythema, premature aging, photodermatosis, and skin cancer development. Phenolic compounds, flavonoids, and hydroxyxicamidic acid esters absorb UV light from the epidermis to protect plant tissues from harmful ultraviolet radiation. The importance of phenolic compounds as sunscreens was investigated by measurement of chlorophyll fluorescence to estimate epidermal UV transmission and the relationship between epidermal UV radiation and the flavonoid content of secondary phenolic metabolites (mainly represented by quercetin glucoside) enriched in apples. UVB component of solar radiation (Nunes, 2018).

One of the plants that contain high polyphenol compounds is Melastoma malabathricum L. (Susanti et al., 2007). Melastoma malabathricum is a plant used in traditional Malay, Indian, and Indonesian medicine to treat diseases including cytotoxicity, antiviral activity, diarrhea, dysentery, vaginal discharge, hemorrhoids, wound infections, preventing the formation of smallpox scar tissue, and hemorrhoids (Azliza et al., 2012; Joffry et al., 2012; Norha et al., 2009). Based on previously reported research, the identification of phytochemicals of M. malabathricum L. is salicilic acid, cyanidin-3-glucoside, cyanidin-3,5-glucoside, malvidin3,5-glucoside, sitosterol, ursolic acid, 2-hydroxyursolic acid, gallic acid, kaempferol, kaempferol3Oequn, quercetin, and rutin (Joffry et al., 2012).

Solar products are for the prevention of UVB radiation and are expressed in terms of sun protection factor (SPF). SPF is the UV energy required to produce the minimum erythema dose (MED) on protected skin, divided by the UV energy required to create a MED on unprotected skin. MED was defined as the shortest time interval or the dose of UV irradiation sufficient to produce minimal visible erythema on unprotected skin (Guyer et al., 2003). The higher the SPF value is, the more efficient it is, and it provides the highest protection against sunburn. Sunscreen products derived from natural ingredients have a better level of safety than those containing synthetic ingredients. Medicinal plants containing antioxidants, phenols, and glycosides can absorb ultraviolet light and are potential sources of sunscreen to be developed (Kumar et al., 2019). Prasiddha et al. (2016) reported on the photoprotector properties possessed by phenol group compounds through electron resonance events in the double bond after interacting with ultraviolet light.

Melastoma malabathricum L. has been reported as a medicine that is antilucre, antiinocceptive, anti-inflammatory, anticarcinogenic, antidiabetic, gastroprotective, hepatoprotective, and able to stimulate the male reproductive system (Balamurugan et al., 2012, 2013, 2015; Kamisan et al., 2013; Kumar et al., 2013; Zabidi et al., 2012; Zakaria et al., 2016a, 2016b). However, studies related to the use of this plant as a source of sunscreen protection have not been investigated. According to the polarity of the solvent used, the separation method is fractionation, which can show the bioactivity approach in discovering natural products from natural ingredients. In this case, the procedure is carried out based on the chemical compounds’ solubility, which is separated from the crude ethanol extract of the M. malabathricum L. plant. The sample in this study is a soluble compound in polar solvents, namely crude extract, ethyl acetate fraction, ethanol, and residue fraction. Zakaria et al. (2011) reported that extract chloroform of M. malabathricum has the slightest radical scavenging activity with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) method compared to aqueous and methanol extracts, which is 17.9 ± 0.2%, where at the same concentration, aqueous and methanol extracts have radical scavenging activity with DPPH method, i.e., 69.8 ± 6.1% and 97.3 ± 0.1%, respectively. Samsu et al. (2020) reported that the n-hexane fraction of the Kersen fruit (Muntingia calabura L.) has the smallest SPF value compared to ethanol extract, ethyl acetate fraction, and ethanol fraction is 3.89 category SPF minimal protection at concentration 800 µg/ml. Chemical content screening is carried out in each solvent used, and quantitative analysis of compound content using HPLC-RP, antioxidant activity tests, and SPF values can be carried out. A step in discovering compounds that can protect the skin from UV rays and become a source of cosmetics with the best SPF value needs to be taken.

In this study, the compound was separated by polarity by liquid–liquid extraction. A thin layer of chromatography was used to identify the compound, quantitative analysis of gallic acid and quercetin in sample compound content with reversed-phase high-performance liquid chromatography (RP-HPLC) and examined the oxidative activity using DPPH and ferric reduction antioxidant power (FRAP) methods. Sun protection is determined by the SPF value. The test result obtained samples that have the potential to be used as raw materials for cosmetic sources that have the best antioxidant activity and SPF value.

MATERIAL AND METHODS

Material

Oven (Modena), camera (Canon), rotary evaporator (Butchi), microscope (Olympus CX51), ultrasonicator (Branson 1510), spectrophotometer UV/Vis (Shimadzu), HPLC (Shimadzu Corporation), and UFLC (Whatman) were used.

Chemicals

Ethanol (Merck), ethanol (Brataco), ethyl acetate (Brataco), aquadestilata, methanol (Merck), H₂O₂ (Merck), formic acid (Merck), acetonitrile (Merck), ortho-phosphoric acid 85% (Merck), HPO₃₂H₂O (Merck), NaHPO₃₂H₂O (Merck), HCl (Merck), AlCl₃ (Merck), FeCl₃ (Merck), metal Mg, H₂SO₄ (Merck), KOH (Merck), gallic acid (Merck), quercitin (Merck), TPTZ (Merck) reagent, ascorbic acid (Merck), and DPPH (Merck) were used.

Methods

Collection and authentication of plant

Melastoma malabathricum L. was collected from Pontianak, West Borneo, Indonesia. Authentication by the Faculty of Biology at Tanjungpura University.
Microscopic analysis

The microscopic study was performed according to Pharmacopeia Herbal Indonesia, 2008. Melastoma malabathricum L. leaf powder was placed on a slide and then dripped with chloral hydrate reagent solution. Tissue in the plant M. malabathricum L. was viewed under a microscope at 10× and 40× magnification.

Extraction

Dried and powdered leaves of M. malabathricum L. were extracted with 78% ethanol from the maceration method. The mash was rotated to obtain the crude extract. Next, the crude extract is separated using a separatory funnel with solvents of different polarities: n-hexane, chloroform, ethyl acetate, and water (Syamsu et al., 2020). We obtained a solvent-insoluble solid layer during the separation process, which was then dissolved with ethanol. We received n-hexane, chloroform, ethyl acetate, ethanol fraction, and residue, namely fraction. The dry fraction is stored at room temperature. The sample in this study is ethyl acetate, ethanol fraction, and residue.

Preliminary phytochemical screening and thin-layer chromatography (TLC)

Preliminary phytochemicals presence of secondary metabolites test-based standard procedure in Health Ministry Republic of Indonesia. According to standard procedures, phenol, flavonoid, alkaloid, steroid, anthraquinone, tannin, and saponin tests are carried out according to standard procedures (Health Ministry Republic of Indonesia, 2008). Secondary metabolit confirmation assay with TLC. Identification with stationary phase is silica gel 60 F254, and the mobile phase is n-hexane : ethyl acetate : formic acid, for comparison: 10 ml : 10 ml : 8 drops. The punctual bystanders in this study are AlCl3, FeCl3, 10% H2SO4, and 10% vanillic sulfuric acid (Bladt, 2009).

RP-HPLC analysis quercetin and gallic acid

The sample in this study is the crude extract, ethyl acetate, ethanol fraction, and residue. An example is compounds that are soluble in polar solvents and dissolved in methanol. After that, it was filtered through a filter paper and a 0.2 µm membrane filter (Whatman). The sample was injected directly into the HPLC system. A standard stock solution of gallic acid and quercetin was prepared in acetonitrile. A standard solution of gallic acid was prepared by dissolving an accurately weighed amount of 5.5 mg in 10 ml acetonitrile as stock solution and making the different solution as calibration curve in the range of concentration 1–75 µg/ml. A standard quercetin solution was prepared by dissolving an accurately weighed amount of 5.5 mg in 10 ml of acetonitrile as stock solution and making different calibration curves in the range of concentration 3 µg/ml–18 µg/ml. All the standard solutions were filtered through a 0.2 µm membrane filter (Whatman) before injection into the HPLC system.

Chromatography condition in RP-HPLC

Chromatography condition method was used according to Ravichandran et al. (2010), with modifications. HPLC analysis was performed using Shimadzu Corporation UFLC equipped with reservoir tray, Prominence Degasser (DGU-20A5), Prominence Liquid Chromatography (LC-20AD), Prominence Communication Bus Mobile (CBM-20A), Prominence UV Detector (SPD-20A), and Colom Oven (CTO-20 A). VP-ODS shim-pack C-18, 250 x 4.6 (mm), reverse phase column packed with 4.5 µm diameter particles with the mobile phase consisting of acetonitrile-0.05% phosphoric acid with pH 3.42 (40:60, v/v) for gallic acid, and quercetin with a mobile phase composed of acetonitrile-phosphate buffer with pH 5.4 (40:60, v/v). The mobile phase was filtered through a 0.2 µm membrane filter (Whatman) and then deaerated ultrasonically before use. The temperature was maintained at 30°C, with an injection volume of 50 µl and a 1 ml/min flow rate. The detection was monitored at 263 nm for gallic acid and 370 nm for quercetin with a UV detector (Ravichandran et al., 2010).

Antioxidant activities with DPPH and FRAP method

The DPPH antioxidant activity test was used to test the free radical scavenging activity of the sample. The technique was used in this study according to Gülçin (2012) and Kikuzaki et al. (2002), with minor changes. 1 ml of each sample with different concentrations (0.5–250 ppm) add 3 ml of 1 mM DPPH and 1 ml of methanol. It was incubated for 15 minutes at room temperature in a dark place, and the absorbance was measured at 515.5 nm. A triplicate experiment was performed with gallic acid, quercetin, and vitamin C as comparison compounds in this study (Gülçin, 2012; Kikuzaki et al., 2002).

Antioxidant activity testing with the FRAP method was performed according to Syamsu et al. (2017), with minor modifications. 30 µL of each sample at different concentrations 1 to 650 µg/ml added 30 µl FeCl3 solution (3 mM in 5 mM citric acid) and 240 µl TPTZ reagent (1 mM in 0.05 M HCL) in a 96-well microplate. Incubation was performed for 20 minutes at room temperature, and absorbance was measured at 615 nm. Absorbance was read with Elisa Reader (Syamsu et al., 2017). The experiment was performed in triplicate using gallic acid, quercetin, and vitamin C as comparative compounds.

Identification in vitro SPF

The sample in this study was dissolved in ethanol. The concentration was 250 µg/ml and scanned in the range of 290–320 nm (at 5 nm intervals). Screening sun protection activity was measured by determining SPF in vitro based on the following equation proposed by Fonseca and Rafael (2013):

\[
SPF = CF \sum_{i=1}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda),
\]

where CF is the correction factor, EE is the erythema effect in the spectrum (nm), I is light intensity (nm), and Abs is the absorbance sample. The absorbance sample was measured thrice and was used for SPF calculation.

Statistical analysis

Data are presented as means ± standard deviation. Statistical analysis was carried out using SPSS software to analyze ANOVA, followed by a post hoc test to see differences between groups. Statistical significance with a value of p < 0.05 was considered significant.

RESULTS AND DISCUSSION

Morphology and microscopic of M. malabathricum L.

Melastoma malabathricum L. is a wild plant that grows in shrubs, on mountain slopes, and at an altitude of 1,650 m above
sea level with good sunlight. The plant is bushy, with a vertical plant height of 0.54 m, branched, scaly, and hairy. The leaves are single, petiolate, opposite each other, oval-shaped, elongated to oval, pointed tip, rounded base, flat edge, short-haired surface, sparse, and stiff so that it looks rough. Flowers are arranged at the ends of the branches, with red-purple color and tiny brown seeds (Joffry et al., 2012).

The plant morphology of *M. malabathricum* L. can be used as a source of authentication, as shown in Figure 1 and Table 1. Microscopy of the tissue *M. malabathricum* L. We characterized the microscopic appearance of *M. malabathricum* L. leaf powder. Known cell type pericellular stomata, unicellular outer hairs, spiral transport bundles, and ca-oxalate crystals were contained in transport bundle, parenchyma, and collenchyma cell as shown in Figure 2.

**Percentage yield of extract and fraction**

Extraction was carried out on the *M. malabathricum* L. implant by maceration with 78% ethanol to obtain a thick extract, followed by separation by liquid–liquid extraction to get n-hexane, chloroform, ethanol, ethyl acetate, and residue fractions. The yield of extract and fractions of *M. malabathricum* L. is shown in Table 2.

**Preliminary phytochemical screening and TLC**

Phytochemical prescreening performs tests for the qualitative determination of the content of secondary metabolites. The result is shown in Table 3. Confirmation test of secondary metabolites of *M. malabathricum* L. leaf extract and fraction by TLC is shown in Figure 3, and a comparison of retention factor (RF) in each sample is explained in Table 4.

A preliminary chemical component test was performed, examining phenolic compounds, flavonoids, alkaloids, triterpenoids, steroids, quinones, tannins, and saponins. The results are shown in Table 3. The following study is a confirmatory test of the chemical content of the samples performed by TLC using the mobile phase n-hexane : ethyl acetate : formic acid (10 ml : 10 ml : 8 drops). The results are the crude extract, ethyl acetate, and ethanol fraction containing gallic acid and quercetin. The point reagent is used with 10% AlCl$_3$, and 10% FeCl$_3$ is sprayed to obtain blue and yellow colors. The elution by chromatographic separation of gallic acid and quercetin was 0.15 and 0.43, respectively, as marker compounds. Samples were obtained by elution separation of the crude extract, ethyl acetate, and ethanol fraction of 0.15 and 0.38, respectively. According to the research of Karupiah and Ismail (2013), quercetin was known to be contained in the *M. malabathricum* leaf extract, which was extracted with Soxhlet in chloroform, ethyl acetate, and methanol as solvents (Bladt, 2009). The samples’ separation elution (RF) is shown in Figure 3, and the comparative model and the marker compound are shown in Table 4. This study is in line with Giri and Rajbhandari (2018) and Nozlena et al. (2018), which reported that secondary metabolites in the methanol extract are phenols, flavonoids, tannins, saponins,
and steroids, and the ethyl acetate extract contains gallic acid, quercetin, tannins, quinones, and saponins.

**RP-HPLC analysis**

Analysis using RP-HPLC the initial stage is to determine the mobile phase. The mobile phase used is acetonitrile–0.5% phosphoric acid with pH 3.42 (40 : 60, v/v) for gallic acid and quercetin with the mobile phase consisting of acetonitrile–phosphate buffer with pH 5.4 (40 : 60, v/v). The separation of gallic acid and quercetin as markers is shown in Figures 4 and 5. The chromatogram of the separation results in the samples in this study is shown in Figures 4 and 5. The study results in Figures 4 and 5 show that the model has several chromatogram peaks. There are other compounds besides gallic acid and quercetin, while the gallic acid and quercetin compounds have one chromatogram peak with a retention time (RT) value of 2.82 and 5.85, respectively. In line with Karupiah and Ismail’s (2013) study analysis, chromatogram peak quercetin with RP-HPLC with RT value is 5.0, and analysis study chromatogram peak gallic acid with RP-HPLC RT value is 5.29 and 8.5 min (Fernandes, 2015; Patel et al., 2010).

**Table 1. Morphology characteristics of *Melastoma malabathricum* L. leaves.**

| Parameters                  | Observation study                                      |
|-----------------------------|--------------------------------------------------------|
| Colour                      | Green                                                  |
| Shape                       | Oval                                                   |
| Size                        | Length: 9.7 cm; width: 2.85 cm                         |
| Apex                        | Tapered                                                |
| Texture                     | Stiff and rough                                        |
| Base                        | Scaly and hairy                                        |
| Arrangement of leaves       | Opposite                                               |
| Flower                      | Compound with a reddish-purple color                   |
| Seed                        | Small with brown color                                 |

**Table 2. Percentage of the yield of extracts of *Melastoma malabathricum* L. leaves.**

| Sample                    | Percentage yield (%w/w) |
|---------------------------|-------------------------|
| Crude extract             | 10.8                    |
| Ethanol fraction          | 13.78                   |
| Ethyl acetate fraction    | 7.99                    |
| Residue                   | 59.29                   |

**Figure 2.** Powder Characteristics with a Microscope of *Melastoma malabathricum* L. Leaves (x400).
Table 3. Screening of secondary metabolite content in crude extract and fractions from *Melastoma malabathricum* L. leaves.

| Secondary metabolite | Crude extract ethanol 78% | Ethanol fraction | Ethyl acetate fraction | Residue |
|----------------------|----------------------------|------------------|------------------------|---------|
| Phenol               | +++                       | +++              | +++                    | +++     |
| Flavonoids           | +                         | +                | +                      | +       |
| Alkaloids            | -                         | -                | -                      | -       |
| Terpenoids           | -                         | +                | -                      | -       |
| Steroids             | -                         | -                | -                      | -       |
| Anthraquinones       | -                         | -                | -                      | -       |
| Tannins              | +                         | -                | +                      | +       |
| Saponins             | +                         | -                | -                      | +       |

Key: - = absent; + = present in small amount; ++ = present in moderate quantity; +++ = present in large quantity.

**Figure 3.** Separation sample with thin layer chromatography, mobile phase n-hexane: Ethyl acetate: Formic acid (10 ml: 10 ml: 8 drops) and stationary phase silica gel F254 sample volume = 10μL; Gallic acid and Quercetin Volume = 8μL and 4 μL. Key: 1. Ethanol fraction; 2. Ethyl acetate fraction; 3a. Gallic acid; 3b. Quercetin; 4. Crude extract ethanol 78%.
Table 4. Comparison between RF value of crude extract, ethyl acetate fraction and ethanol fraction, and gallic acid and quercetin standards.

| No | Name of sample          | RF Value   |
|----|-------------------------|------------|
|    |                         | Sample | Gallic acid | Sample | Quercetin |
| 1  | Crude extract           | 0.15    | 0.15        | 0.380  |           |
| 3  | Ethyl acetate fraction  | 0.15    | 0.15        | 0.380  | 0.432     |
| 4  | Ethanol fraction        | 0.15    | 0.388       |        |           |

Figure 4. HPLC Chromatogram marker compound gallic acid with sample crude extract and its fraction of Melastoma malabathricum L. mobile phase phosphoric acid 0.05% pH 3.42-acetonitrile (60:40) flow rate 1ml/min
**Figure 5.** HPLC Chromatogram marker compound quercetin with sample crude extract and its fraction of *Melastoma malabathricum* L. mobile phase phosphate buffer pH 5.4-acetonitrile (60:40) flow rate 1 ml/min
Quantitative analysis of gallic acid and quercetin with RP-HPLC

This study’s determination of gallic acid and quercetin content begins with determining the correlation value of marker compounds (linearity), inter- and intraday precision, accuracy, Limit of Detection (LOD), and Limit of Quantification (LOQ). Linearity method use standard of gallic acid and quercetin using 6 concentration variations with a concentration range of 1–75 g/ml (gallic acid) and 3–18 g/ml (quercetin) values obtained $R^2 = 0.9745$ (gallic acid) and $R^2 = 0.9568$ (quercetin) and the equation $y = 69,520x + 617,132$ and quercetin $y = 134,274x + 257,707$. The standard curves in this study are shown in Figures 6 and 7. Intraday and interday precisions for RT and peak were determined in this study. The repeatability expressed as a percent Relative Standar Deviation (RSD) value (%RSD) in the peak area and RT obtained intraday precision on gallic acid and quercetin compounds was 0.6%–7.3% (peak areas); 0.39%–1.41% (RT), and 0.31%–1.67% (peak area); 1.88%–6.05%. Interday precision for gallic acid and quercetin is 0.34%–4.44% (peak area), 0.22%–5% (RT), 0.31%–1.66% (peak area), and 0.1%–2.34% (RT); intraday and interday precision measurements can be seen in Tables 5 and 7.
Detection limits were evaluated based on the signal-to-noise ratio expressed as LOD and LOQ. Result in this study are $1.15 \times 10^{-6}$ µg/ml, $3.84 \times 10^{-6}$ g/ml, and 0.2 g/ml and presented in Tables 5 and 7. The measurement accuracy is expressed in percent recovery, the values for gallic acid compounds are 99.2%–100.53%, and for quercetin, compounds are 98.87%–100.84%. The measurement results are shown in Tables 6 and 7. The results of measurements on samples known to contain gallic acid and quercetin can be seen in Table 9. In Table 9, it can be seen that the highest gallic acid content is found in the ethyl acetate fraction with $p > 0.05$, different significantly from the three samples. On the contrary, the quercetin content is not significantly different among the four samples with $p > 0.05$ with a content of 0.03–0.93 mg/g sample.

**Antioxidant activity DPPH and FRAP methods**

Investigation of the antioxidant activity in extracts and fractions of *M. malabathricum* L. leaves was performed using DPPH and FRAP methods. The ability of a sample to donate hydrogen and act as a radical scavenger is the DPPH method. One model that converts hydrogen peroxide to hydroxyl radicals is an antioxidant used to reduce iron, namely the iron-reducing...
Table 8. Determination accuracy of quercetin.

| No | Concentration (%) | Accuracy (%) | RSD |
|----|-------------------|--------------|-----|
| 1  | 80                | 100.84       | 99.79 | 99.36 | 0.76 |
| 2  | 100               | 98.87       | 100.65 | 100.47 | 0.97 |
| 3  | 120               | 100.15       | 99.59 | 100.26 | 0.36 |

Table 9. Gallic acid and quercetin content of crude extract and fractions of *M. malabathricum*.

| Sample                  | Retention Time (RT) | Gallic Acid (mg/g) | Retention Time (RT) | Quercetin (mg/g) |
|-------------------------|---------------------|-------------------|---------------------|-----------------|
| Crude extract           | 2.52                | 10.25 ± 0.59      | 5.58                | 0.40 ± 2.03     |
| Ethanol fraction        | 2.65                | 49.29 ± 0.49      | 5.65                | 0.93 ± 0.06     |
| Ethyl acetate fraction  | 2.52                | 4.63 ± 0.14       | 5.60                | 0.86 ± 2.99     |
| Residue                 | 2.48                | 7.54 ± 0.61       | 5.70                | 0.03 ± 3.74     |

Table 10. Antioxidant activity of *M. malabathricum* extract, fractions and residue, and standard compounds.

| Sample                  | Antioxidant activity (IC₅₀) µg/ml | DPPH | FRAP |
|-------------------------|----------------------------------|------|------|
| Crude extract           | 6.8 ± 0.69                       | 16.6 ± 2.7 |      |
| Ethanol fraction        | 1.9 ± 0.12                       | 4.2 ± 1.5 |      |
| Ethyl acetate fraction  | 3.7 ± 1.5                       | 31.9 ± 1.9 |      |
| Residue                 | 9.3 ± 1.07                       | 38.96 ± 5.2 |      |
| Gallic acid             | 1.69 ± 0.21                      | 4.04 ± 0.55 |      |
| Quercetin               | 5.21 ± 0.98                      | 12.9 ± 0.26 |      |
| Ascorbic acid           | 1.9 ± 0.2                        | 3.13 ± 0.86 |      |

Table 11. SPF of *M. malabathricum* extract, fractions and residue, and standard compounds.

| Sample                  | SPF value (at 250 ppm) |
|-------------------------|-----------------------|
| Crude Extract           | 28.9 ± 0.6            |
| Ethanol fraction        | 59.3 ± 0.93           |
| Ethyl acetate fraction  | 17.5 ± 0.5            |
| Residue                 | 13.2 ± 0.3            |
| Gallic acid             | 67.86 ± 4.40          |
| Quercetin               | 123.48 ± 14.38        |

Antioxidant power (FRAP) method. *Melastoma malabathricum* L. antioxidant research study is shown in Table 10. Comparative compounds used in this study were gallic acid, quercetin, and vitamin C. The test results using the DPPH method were analyzed statistically with a p-value < 0.05. It is known that the ethanol and ethyl acetate fractions have antioxidant abilities that are not significantly different from gallic acid, ascorbic acid, and quercetin. The test results using the iron reduction method were analyzed by statistical analysis of p < 0.05. It was found that the ethanol fraction, residue, crude extract, and ethyl acetate fraction had antioxidant abilities that were not significantly different from gallic acid, ascorbic acid, and quercetin, and the ethyl acetate fraction has the highest training comparable to vitamin C and quercetin standard with IC₅₀ of ethyl acetate, which is 1.9 ± 0.12 µg/ml (DPPH) and 4.2 ± 1.5 µg/ml (FRAP).

Determination of the activity of each sample based on the test shows that there are differences in antioxidant capacity in different test methods. Testing with the iron reduction method (FRAP), the sample solution provided antioxidant activity with the Fenton reaction mechanism by chelating Fe²⁺ metal ions that we are able to convert hydrogen peroxide into hydroxyl radicals on the skin. In this study, a solution of FeSO₄₂± is equivalent to Fe²⁺. According to research conducted by Kulisic et al. (2004) and Lin et al. (2008), flavonoid and phenol group compounds can contribute to metal ion chelation and reduce the formation of hydroxyl radicals from superoxide radical anions. Zakaria et al. (2011) reported that the total phenolic content and antioxidant activities in aqueous, chloroform, and methanol extract showed that aqueous and methanol extracts exhibit high antioxidant capacity at 500 µg/ml is 69.8% and 97.3% with DPPH radical scavenging methods, respectively. The linear correlation between high total phenolic content and antioxidant activity was reported in a previous study by Wu et al. (2006).

According to Choquenet et al. (2009) and Saewan and Jimtaisong. (2013), polyphenolic compounds and flavonoids are candidates for photoprotective products. Phenolic compounds are known to act in redox-sensitive signaling cascades to inhibit DNA damage. They help protect against UV-induced generation of oxygen-free radicals and lipid peroxidation. The high content of flavonoids in plants can also save the revolutionary oxygen-free era caused by UV rays (Saewan and Jimtaisong., 2013). The study results of the acetone fraction in A. Dying plants have high phenol and flavonoid content. They can protect against UV rays (SPF capability) which may be a cosmetic for sun protection. Quercetin is a compound that has been tested and has the potential as a topical SPF in humans and can protect against UVA and UVB.
rays (Choquenet et al., 2008). In line with research conducted by Amini et al. (2019), a positive relationship was found between the value of DPPH radical protection activity (IC$_{50}$), total phenol content, and total flavonoids with an SPF value.

Effect of *M. malabathricum* L. extract and fraction on SPF Value

The screening activity of sunscreen was measured by determining the SPF in vitro. The results of this study are presented in Table 10. SPF is a quantitative measurement in measuring the effectiveness of sun protection products. Adequate protection against sunburn and skin problems caused by the sun. Protective effects must have the ability to absorb light at a wavelength in the range of 290–400 nm. The evaluation used is in vitro by measuring the SPF value, which is the initial stage to screen potential products. The results of the measurements carried out in this study are presented in Table 11. The results of the SPF measurement at a concentration of 250 µg/ml were 13.2 ± 0.3 to 59.3 ± 0.9, where the ethyl acetate fraction had the highest value with a significance value of $p < 0.05$, but not as good as with the comparison compounds used were quercetin and gallic acid with SPF values of 123.48 ± 14.38 and 67.86 ± 4.40. The crude extract result from this study, in line with Abd Gani et al. (2019), who showed that the SPF value of ethanol extract is 28.9 ± 0.6 and 22.44 ± 0.03 with antiscavenging DPPH methods. From the evaluation results by doing fractionation in this study using polar solvents that are ethyl acetate, ethanol, and aqueous. Significantly, the ethyl acetate fraction has the highest SPF value compared to crude extract before fractionation; the results of the study can be seen in Table 11. It is known that separating the crude extract can increase the SPF value, so it is crucial to do so that it can be used in the manufacture of product preparations with a higher value SPF as a sunscreen protector preparation.

Gallic acid is commonly used in industry as an additive in food and cosmetics. The ability of gallic acid as a cell protector has been proven in in vivo studies that have been carried out, which have anticancer activity through the induction of cell apoptotic events. The ability of gallic acid to protect against oxidative damage to biological systems such as hydroxy, superoxide, and peroxyl. Protection against lipid peroxidation events through the mechanism of free radical scavenging activity and lipid peroxidase inhibitory activity (Badhani et al., 2015). The flavonol quercetin is believed to protect against damage caused by UV radiation in plants (Fahlman and Krol, 2009). The ability of quercetin to protect plants from UV damage has been demonstrated by Rozema et al. (2002), Liu et al. (2013), and Solovchenko and Schmitz-Eiberger (2003) on *Vicia faba* (fava beans), *Brassica napus* (canola), and *Malus domestica* (apples, *Braeburn cultivars*) plants from the study believed to be responsible for preventing damage caused by UV radiation. The ability of quercetin-containing products to be applied topically from the quercetin aglycone has been shown to be effective in avoiding UVC radiation-induced liposome peroxidation (Pandel et al., 2013), as well as preventing UVB-induced myeloperoxidase activity, glutathione depletion, and proteinase secretion, which are overall markers of oxidative stress (Casagrande et al., 2006).

CONCLUSION

The results of this study show that gallic acid levels in crude extract, ethyl acetate, ethanol fraction, and residue are 10.25 ± 0.59 mg/g, 49.29 ± 0.49 mg/g, 6.43 ± 0.14 mg/g, and 7.54 ± 0.61 mg/g, respectively, and quercetin content in crude extract, ethyl acetate, ethanol fraction, and residue was 0.40 ± 2.03 mg/g, 0.93 ± 0.06 mg/g, 0.86 ± 2.99 mg/g, and 0.03 ± 3.74 mg/g, respectively. Antioxidant activity from DPPH and FRAP assay showed that the ethyl acetate fraction has the highest training compared to vitamin C and quercetin standard; IC$_{50}$ of ethyl acetate is 1.9 ± 0.12 µg/ml and 4.2 ± 1.5 µg/ml, respectively. Identification of SPF result ethyl acetate fraction with the highest value is 59.3 ± 0.9. The result showed that ethyl acetate fraction has the potential to be a raw material for the formulation of sunscreen preparations with SPF values included in the ultracategory. It concludes that ethyl acetate fraction of *M. malabathricum* L. leave can be natural resources for sunscreen development products.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

his study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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