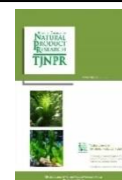
Trop J Nat Prod Res, January 2023; 7(1):2172-2177 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article



Potential Extracts of Melastomataceae Species from Mount Merapi National Park as Sunprotection Material with Antioxidation and Antiglycation Activities

Sendy Junedi[1], Ari Nurwijayanto[2], Desy D. Simamora[1], Angela M. Palimbongan[1], Ines S. Arsiningtyas[1]*

[1]*Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta, Yogyakarta 55281, Indonesia.*
[2]*Mount Merapi National Park, Directorate General of Nature Resources and Ecosystem Conservation, Ministry of Environment and Forestry, Yogyakarta 55582, Indonesia.*

ARTICLE INFO

Article history:
Received 30 November 2022
Revised 06 January 2023
Accepted 09 January 2023
Published online 01 February 2023

Copyright: © 2023 Junedi *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Ethanol extracts from the leaves of *Clidemia hirta* (L.) D. Don., and *Melastoma affine* D. Don., growing in Mount Merapi National Park, Indonesia, had high antioxidant activity similar to ascorbic acid. Natural substances with high antioxidant activity potentially inhibit UV-induced skin photoaging. This study aimed to investigate the sun protection factor (SPF) and antiglycation activity of *C. hirta* and *M. affine* ethanol extracts to identify and obtain candidate photoprotective ingredients for sunscreen products. Proximate analysis of extract simplicial was performed for standardization, and their secondary metabolites quantified to understand their bioactivities. Flavonoids, alkaloids, and saponins were quantified using the aluminum chloride, bromocresol green, and vanillin-sulfuric acid methods, respectively. Phenolic compounds and tannins were quantified using the Folin-Ciocalteu method. The *C. hirta* extract showed a higher SPF value in UV spectrophotometry, and antiglycation activity through bovine serum albumin-glucose (BSA-glucose) method, than the *M. affine* extract. An antioxidant assay using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method confirmed that the *C. hirta* and *M. affine* extracts were more potent and had slightly lower antioxidant activity, respectively, than ascorbic acid. The high bioactivity of *C. hirta* extract was likely due to phenolic compounds, flavonoids, and tannins, because these metabolites were more abundant in *C. hirta* extract than in *M. affine* extract. These results indicated that the ethanol extract of *C. hirta* leaves has the potential to be developed as an ingredient of sunscreen products for skin photoprotection.

Keywords: Antiglycation, Antioxidation, *Clidemia hirta*, *Melastoma affine*, Phytochemical properties, Sun protection factor.

Introduction

The ultraviolet (UV) radiation index in Indonesia is 5–10 during bright sunshine from 10 AM to 2 PM, as recorded by the Indonesia Meteorology, Climatology, and Geophysical Agency. According to the World Health Organization, this range is categorized as moderate to very high, representing the effects of UV exposure on the human skin and immune system.[1] Overexposure to UVA (315–400 nm) and UVB (280–315 nm) radiations can trigger oxidation of genetic material in the skin epidermis and dermis. Reactive oxygen species (ROS) from UV exposure cause gene mutations, which increase the risk of non-melanoma and melanoma. [2,3]
UV-induced ROS also generates nonenzymatic glycation or oxidation of proteins, lipids, and nucleic acids.[4] These reactions produce advanced glycation end products (AGEs) primarily in the extracellular matrix (ECM) proteins of the skin, such as elastin and collagen, leading to stiffening and loss of elasticity.[5] Furthermore, AGEs generate ROS, which damage dermal proteins, trigger inflammatory responses, and induce melanogenesis by binding to its receptor in the melanocytes.[4] All these factors demonstrate the effects of AGEs on the symptoms of photoaging such as skin wrinkling and pigmentation.

*Corresponding author. E mail: ines.septi@uajy.ac.id
Tel: +62-274-487711 ext. 2188

Citation: Junedi S, Nurwijayanto A, Simamora DD, Palimbongan AM, Arsiningtyas IS. Potential Extracts of Melastomataceae Species from Mount Merapi National Park as Sunprotection Material with Antioxidation and Antiglycation Activities. Trop J Nat Prod Res. 2023; 7(1):2172-2177. http://www.doi.org/10.26538/tjnpr/v7i1.14.

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Physical or chemical sunscreen materials are commonly used to avoid contact between the UV rays and the skin. The level of absorption or reflection of UV rays by the sunscreen materials is represented as SPF value; the higher the value, the better the protection from UV rays.[6] Synthetic sunscreen ingredients are commonly available in the market, but once applied, their effective duration is limited; hence, reapplication to the skin every two hours is advised.[7] Moreover, synthetic sunscreen ingredients might get activated by UV rays, and produce photosensitizers that cause adverse reactions in the skin.[8] Due to these reasons and owing to their wide variety of biological activities, the use of naturally occurring substances for photoprotection has gained attention in recent years.
Natural substances such as polyphenols (flavonoids, tannins), carotenoids, anthocyanidins, vitamins, and volatile oils from vegetables, fruits, and medicinal plant parts (leaves, flowers, berries), are more effective in protecting the skin from UV radiation than synthetic chemicals.[9] In addition to their capacity for reflecting UV rays, most of these natural substances have long-term benefits because of their strong antioxidation activity against ROS and other bioactive effects in the skin, such as anti-inflammatory, anti-collagenase, anti-elastase, and immunomodulatory activities.[10–12] Photoaging is built up by multiple mechanisms following UV irradiation, hence addition of natural substances into sunscreen products is a promising strategy because they possess high SPF value, antioxidant activity, and also inhibit ECM damage.
Ethanol extracts from Melastomataceae plants, *Clidemia hirta* (L.) D. Don., and *Melastoma affine* D. Don growing in Mount Merapi National Park, Indonesia, were reported to have high antioxidant activity similar to ascorbic acid, a well-known potent antioxidant.[13] These antioxidant materials in the ethanol extract of *C. hirta*, and *M. affine* leaves have the potential to inhibit ECM damage caused by glycation, since ROS is a major initiator for many mechanisms of photoaging. Furthermore,

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

flavonoids and alkaloids detected previously in the extracts are also expected to absorb UV rays due to the presence of chromophores in their structure.
This study analyzed the ability of the ethanol extracts of *C. hirta* and *M. affine* leaves to inhibit protein glycation, determined their SPF values and reconfirmed their antioxidant activities. To understand the relationship between these three bioactivities and metabolites associated with them, quantitative determination of secondary metabolites (phenolic compounds, flavonoids, tannins, alkaloids, and saponins) was performed. The aim of this study was to identify the SPF values, antiglycation, and antioxidant activities of *C. hirta* and *M. affine* extracts to find potential natural materials that could be candidates for photoprotection ingredients in sunscreen products.

Materials and Methods

Materials
Atropin sulfate was obtained from Indonesian FDA Laboratory Services, Jakarta, Indonesia. Ethanol, methanol, chloroform, Folin-Ciocalteu reagent, aluminum chloride, potassium acetate, sodium hydroxide, sulfate acid, tannic acid, sodium carbonate, chloride acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), bromocresol green, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dodecahydrate, D-fructose, and D-glucose were procured from Merck (Germany). Quercetin, gallic acid, saponin, and aminoguanidine hydrochloride were purchased from Sigma-Aldrich (Germany). Bovine serum albumin was obtained from HiMedia Laboratories (India). All other reagents were of analytical grade.

Preparation of extracts
Leaves of *C. hirta* and *M. affine* were collected from Mount Merapi National Park, Yogyakarta, Indonesia in June 2021. They were taxonomically identified, by Prof. Purnomo from the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada, Indonesia. Voucher identification number for *C. hirta* and *M. affine* were 0141090/S.Tb./IX/2021 and 0141089/S.Tb./IX/2021, respectively. The samples were dried at 48°C for 24 h and then ground until a dry powder was obtained. Moisture content analysis was performed using a Moisture Analyzer (OHAUS MB120).
Extraction was performed by adding 70% ethanol to 100 g of dried powder with a material to solvent ratio of 1:10 (v/v). The mixture was macerated at 120 rpm and 24°C for 24 h. The residue was filtered. The filtrate was concentrated using a vacuum rotary evaporator (IKA® RV 10 Basic) at 60 rpm and 50°C . The concentrated extract was dried in an oven (MMM Medcenter Venticell) at 45°C and weighed to calculate the yield (%). The extracts were stored at 4°C until further analysis.

Proximate analysis
Determination of total ash content and acid insoluble ash content
Two grams of dried powder from each plant sample was incinerated in a silica crucible until no smoke or charcoal remained. The charred material was weighed and burned at 800°C for 6 h. The total ash content was expressed as %w/w.[14]
The total ash was boiled in 2 N hydrochloric acid (HCl) for 5 min and filtered. The filter papers were rinsed with hot water and the residue along with the filter paper was burned at 800°C for 6 h and weighed. The total acid-insoluble ash was expressed as %w/w.[14]

Determination of water- and ethanol-soluble extract contents
Five grams of dried powder from each plant sample was macerated separately with 100 mL of absolute ethanol and water-saturated chloroform at 120 rpm and 24°C for 6 h, followed by maceration for 18 h without shaking. The residue was filtered. The filtrate was concentrated using a vacuum rotary evaporator at 60 rpm and 50°C. The concentrated extract was dried in an oven at 45°C and weighed to calculate yield in percentage.[14]

Phytochemical analysis
Determination of total flavonoid content
Extracts were prepared at a concentration of 5,000 mg/L using 50% ethanol. The concentration of quercetin, used as a standard chemical was prepared at 250–1,000 mg/L. Tested sample (0.5 mL) was mixed with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium and 1.5 mL of absolute ethanol. Distilled water was added to this mixture to obtain a final volume of 5 mL, and allowed to stand for 30 min at room temperature (25°C). Absorbance was measured at 415 nm. The total flavonoid content was calculated as quercetin, based on the calibration curve. The results were expressed as milligrams quercetin equivalent (QE)/g extract. The analysis was performed in triplicates.[14]

Determination of total phenolic content
Extracts were prepared at a concentration of 5,000 mg/L using 50% ethanol. The concentration of gallic acid used as a standard chemical was–10–200 mg/L. Each tested sample (1 mL) was added to 5 mL of 7.5% Folin-Ciocalteu solution and incubated for 8 min at room temperature (25°C). After incubation, 4 mL of 1% NaOH was added and the mixture was incubated for 1 h at room temperature (25°C). in the dark. Absorbance was measured at 730 nm. The total phenolic content was calculated as gallic acid from the calibration curve, and the results were expressed as milligrams of gallic acid equivalent (GAE)/g of extract. The analysis was performed in triplicates.[14]

Determination of total tannin content
The extracts were prepared at a concentration of 5,500 mg/L using 50% ethanol. The concentration of tannic acid used as a standard chemical was–55–220 mg/L. Each tested sample (0.5 mL) was added to 2.5 mL of 10% Folin-Ciocalteu solution and 2.5 mL of 7.5% Na_2CO_3. The mixture was incubated for 1 h. Absorbance was measured at 775 nm. The total tannin content was calculated as tannic acid from the calibration curve, and the results were expressed as milligrams of tannic acid equivalent (TAE)/g of extract. The analysis was performed in triplicates. [15,16]

Determination of total alkaloid content
The extracts were prepared at a concentration of 5,000 mg/L with 2 N hydrochloric acid and then filtered. The concentration of atropine sulfate used as a standard chemical was 20–100 mg/L. Each tested sample (1 mL) was transferred to a separating tunnel, and 5 mL of phosphate buffer pH 4.7 was added, followed by 5 mL of 10^{-4} M bromocresol green solution, and 5 mL chloroform. The mixture was shaken, and the chloroform layer was collected. Absorbance was measured at 415 nm against a blank. The total alkaloid content was calculated as atropine sulfate from the calibration curve, and the results were expressed as milligrams of atropine sulfate equivalent (AE)/g extract. The analysis was performed in triplicates. [17]

Determination of total saponin content
The extracts were prepared at a concentration of 5,500 mg/L with 50% ethanol. The concentration of saponin used as a standard chemical was–50–150 mg/L. Each tested sample (0.5 mL) was reacted with 8% vanillin solution and 4 mL of 72% sulfuric acid. The mixture was incubated at 60 °C for 10 min. Absorbance was measured at 544 nm. Total saponin content was calculated as saponin from the calibration curve and the results were expressed as milligrams of saponin equivalent (SE)/g of extract. The analysis was performed in triplicates. [18]

Determination of SPF value
Extracts were prepared in triplicates at concentrations of 25 mg/L and 50 mg/L with 50% ethanol. The absorbance of the samples was measured at UV-B wavelength of 290–320 nm, at 5 nm increments, with three determinations made at each increment. Quercetin was used as a standard flavonoid with high SPF. SPF was calculated by applying the following equation: [19]

$$\mathrm{SPF} = \mathrm{CF}\sum_{290}^{320} EE(\lambda).I(\lambda).Abs(\lambda) \quad (1)$$

Where CF (correction factor) is 10 (constant), EE is the erythemogenic effect, I is the intensity of the sun, and Abs is the absorbance of the sample. The constants EE and I were pre-defined according to Table 1.

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Evaluation of DPPH free radical scavenging activity
Solution containing 0.4 mM DPPH was prepared in absolute ethanol. This solution (1 mL) was added to the test samples: 0.1 mL each of L-ascorbic acid as the standard (final concentration of 13 mg/L), *C. hirta* extract (final concentration of 1–5 mg/L), and *M. affine* extract (final concentration of 1–10 mg/L). The solutions were incubated for 30 min at room temperature (25 °C). in the dark. Absorbance was measured at 517 nm. DPPH scavenging activity was calculated and expressed as the inhibition percentage using the following equation:

$$\% \text{ inhibition} = \frac{\text{absorbance of negative control- absorbance of tested sample}}{\text{absorbance of negative control}} \text{x } 100\%$$

Note: negative control is the DPPH with solvent
The IC_{50} values were calculated using a regression equation from different concentrations of the tested samples. The analysis was performed in triplicates.[20]

Evaluation of antiglycation activity in BSA-glucose model
Protein glycation was assayed based on the method by Kim and Kim, with modifications.[21] The extracts were dissolved in 50% ethanol to obtain a concentration of 10,000 mg/L. Each extract was diluted with deionized water to obtain a series of concentrations. The sample was added to a solution consisting of bovine serum albumin (20 mg/mL), d-fructose (235 mM), and d-glucose (235 mM) in potassium phosphate buffer (200 mM, pH 7.4). The mixture was incubated at 60 °C for 40 h. Fluorescence intensity was measured using a spectrofluorometer (Shimadzu, RF-6000) at excitation and emission wavelengths of 330 nm and 440 nm, respectively. Aminoguanidine hydrochloride was used as the standard inhibitor.

Statistical analysis
All data were analyzed using Microsoft Excel (version 2016). Linear regression analysis was used to determine the concentration of secondary metabolites and IC_{50} to evaluate antioxidation and antiglycation activities.

Results and Discussion

Proximate analysis and extraction
To obtain a reproducible extract, proximate analysis of simplicial was performed before extraction. The parameters determined in the proximate analysis included moisture content, ash content, acid-insoluble ash, water-soluble extractive value, and alcohol-soluble extractive value. Table 2 shows the results of proximate analysis of *C. hirta* and *M. affine* simplicial. The moisture content of simplicial powder from *C. hirta* and *M. affine* leaves was below 10%, which met the quality standards of simplicial. Low moisture content discourages the growth of bacteria, yeast, and fungi, which prevents the degradation of bioactive phytochemicals during storage. The amount of inorganic impurities and silicate, especially sand, in the leaves of both *C. hirta* and *M. affine* were relatively low, as indicated by the ash content and acid-insoluble ash, respectively.
A similar extractive value for both simplicial were found for water (water-soluble extractive value) and alcohol (alcohol-soluble extractive value), indicating that the polar compounds can be collected using both solvents. To obtain a large range of polar compounds and easier extraction handling, 70% ethanol was used instead of only water or absolute ethanol. The extract of *M. affine* was more solid and dry than that of *C. hirta*; therefore, the yield of *C. hirta* extract was approximately 3.5 times higher than that of *M. affine* extract. The semi-solid state of *C. hirta* extract was probably caused by the water-absorbing compounds present in the extract.

Phytochemical content
Five secondary metabolites, that is, phenolic compounds, flavonoids, tannins, alkaloids, and saponins, were analyzed based on reports of the benzene ring chromophores in phenolic compounds, flavonoids, tannins, and some alkaloids absorbing UV radiation.[9] Flavonoids possess direct and indirect antioxidant properties, and modulate several signaling pathways.[22] Saponin is known as an antioxidant and antiglycation compound.[23,24]
C. hirta extract contained more phenolic compounds, flavonoids, tannins, and alkaloids than *M. affine* extract (Table 3). In contrast, saponin in *M. affine* extract was 2.5 times more than in *C. hirta* extract. Among these metabolites, the amounts of phenolic compounds and saponins were the highest in *C. hirta* and *M. affine* extracts, respectively. The different amounts of metabolites present in the extracts might contribute to their difference in bioactivities with respect to the photoaging mechanism.

Table 1: EE and I constants for the calculation of *in vitro* SPF

λ (nm)	EE(λ) x I(λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

EE(λ), erythemogenic effect of wavelength radiation; I(λ), sun intensity at wavelength; *λ*, wavelength.[19]

Table 2: Characteristics of dried plant materials and their extraction yield

Sample	Moisture content (%)	Ash content (%)	Acid insoluble ash (%)	Water soluble extractive (%)	Alcohol soluble extractive (%)	Extraction yield (%)
Clidemia hirta (L.) D.Don	8.84	5.29	1.29	3.72	2.26	25.34
Melastoma affine D.Don	9.11	8.01	2.15	2.68	2.27	7.15

Table 3: Phytochemical contents of extracts

Sample	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)	Total tannin content (mg TAE/g extract)	Total alkaloid content (mg AE/g extract)	Total saponin content (mg SE/g extract)
Clidemia hirta (L.) D.Don	251.02 ± 12.16	43.74 ± 1.02	73.42 ± 1.10	3.25 ± 0.73	145.45 ± 1.88
Melastoma affine D.Don	113.98 ± 1.56	30.53 ± 1.11	22.58 ± 2.55	1.29 ± 0.26	399.02 ± 7.15

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Determination of SPF value

SPF is a parameter of sunscreen efficacy, defined as the UVB energy required to produce minimum erythemal dose (MED) on protected skin divided by the UVB energy required to produce an MED on unprotected skin. The higher the SPF, the more protection the sunscreen offers against sunburn.[25] The SPF value for screening sun protecting materials is usually determined by an in vitro technique involving measuring the spectral transmittance at UVB wavelengths from 290–320 nm.

In this study, to determine the SPF value the absorbance range of the samples was 0.1–1.5, such that the instrument detector correctly measured light intensity. At concentrations of 25 ppm and 50 ppm, all samples, that is, *C. hirta* extract, *M. affine* extract, and quercetin showed absorbance of 0.1–1.5 at 290–320 nm. Therefore, both concentrations were chosen to compare the SPF values of the three samples. The absorbance of *C. hirta* extract at 290–320 nm was higher than that of *M. affine* extract at the same concentration (25 ppm and 50 ppm). Both extracts had lower absorbance than quercetin (Table 4). Flavanol quercetin effectively absorbs UV rays compared to homosalate (a reference filter established as the FDA standard).[26] Therefore, quercetin was used as a positive control material in this study. The SPF values of the 50 ppm *C. hirta* and *M. affine* extracts were 6.10 and 3.53, respectively (Figure 1). At the same concentration, quercetin had an SPF value of 12.37. Concentrations of the extracts and quercetin were correlated with the SPF value. Results showed that the higher the concentration, the higher the SPF value. Thomas B. Fitzpatrick (1988) categorized the skin into six types based on sun reactivity (sunburn and tan): skin types I, II, III, IV, V, and VI. Skin type VI needs highest MED to get sunburn and tan, while skin type I needs the lowest MED compared to other skin types. The minimum recommended SPF value for skin type VI is 4, V is 5–10, and IV is 6–15.[27] Based on these recommendations, we suggest that 50 ppm *C. hirta* extract will be suitable for skin type IV, V and VI, while 50 ppm *M. affine* extract can be used for skin type VI. To obtain higher SPF values for both extracts, the concentration of the extracts should be increased to more than 50 ppm when used in sunscreen products.

In previous study, analysis of 20 extracts from four antioxidant medical plants showed that SPF had a good correlation with phenolic content, but not with flavonoid content and antioxidation activity.[25] Phenolic content in *C. hirta* extract was 2.2 times higher than in *M. affine* extract. This caused *C. hirta* extract to have about two times higher SPF value than that of *M. affine* extract.

Antioxidation and antiglycation activity

DPPH contains an unpaired valence electron at the nitrogen bridge atom. This stable free radical makes DPPH widely used for the antioxidant assay.[28] Ascorbic acid as a positive control of antioxidation can scavenge DPPH free radical instantaneously.[29] In this study, *C. hirta* extract showed higher antioxidation activity than ascorbic acid (Table 5). *M. affine* extract had slightly lower antioxidation activity than ascorbic acid. These data confirmed previous findings by Nurwijayanto *et al*.[13]

The DPPH scavenging activity of extracts might be carried by hydroxyl groups as proton-donating compounds, which are many in phenolic compounds, some types of flavonoids, and tannins. *C. hirta* extract contained more phenolic compounds, flavonoids, and tannins than *M. affine* extract; therefore, the high antioxidant activity of *C. hirta* was likely caused by these metabolites.

UV-induced AGEs are known to contribute towards skin photoaging, such as skin wrinkling and pigmentation. To prevent the formation of AGEs, inhibition of nonenzymatic glycation is necessary. The anti-glycation BSA-glucose assay in this study represented two steps of a spontaneous non-enzymatic glycation reaction. The first step is the oxidation of glucose and fructose, which leads to the production of ketoaldehyde and superoxide radicals. Ketoaldehyde reacts with the amino groups of BSA-producing amadori products. The second step is the autooxidation of amadori products into active dicarbonyl compounds, which leads to the production of AGEs and radical superoxide. This glycation reaction causes the carboxylation of the cysteine residues of BSA and decreases the reduced thiol content. [30] Aminoguanidine is a well-known synthetic compound which can block the formation of AGEs by interacting with amadori-derived products.[31] Therefore, aminoguanidine was used as a positive control material in the antiglycation BSA-glucose assay.

C. hirta extract had higher anti-glycation activity than *M. affine* extract (Table 5). Both extracts had lower glycation activity than aminoguanidine. Many studies have reported that phenolic and flavonoid compounds in plant extracts are responsible for their antiglycation activity.[32] Polyphenols, flavonoids, and tannins can act at different stages of AGEs formation. They can inhibit Schiff base rearrangement and decrease the formation of amadori products in the first step or cause block oxidation or hydrolysis of amadori products in the second step.

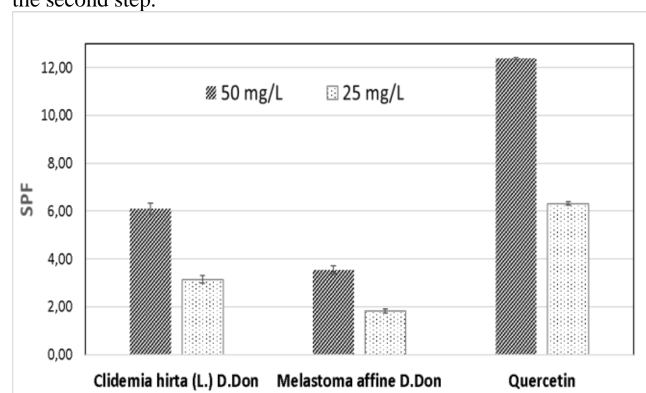


Figure 1: Sun protection factor (SPF) values of extracts calculated through spectrophotometry with quercetin as a positive control. Data given as mean ± standard deviation of triplicate test.

Table 4: Absorbance of extracts and quercetin

Wavelength (nm)	*Clidemia hirta* (L.) D.Don		*Melastoma affine* D.Don		Quercetin	
	50 ppm	25 ppm	50 ppm	25 ppm	50 ppm	25 ppm
290	0.891 ± 0.037	0.451 ± 0.021	0.448 ± 0.019	0.2287 ± 0.010	1.127 ± 0.003	0.573 ± 0.007
295	0.792 ± 0.032	0.402 ± 0.019	0.411 ± 0.018	0.2100 ± 0.010	1.182 ± 0.003	0.602 ± 0.007
300	0.693 ± 0.027	0.355 ± 0.017	0.381 ± 0.017	0.1953 ± 0.009	1.218 ± 0.003	0.621 ± 0.007
305	0.596 ± 0.022	0.307 ± 0.016	0.350 ± 0.017	0.1800 ± 0.009	1.242 ± 0.003	0.634 ± 0.007
310	0.508 ± 0.016	0.265 ± 0.014	0.320 ± 0.017	0.1650 ± 0.009	1.256 ± 0.003	0.641 ± 0.008
315	0.429 ± 0.013	0.227 ± 0.012	0.291 ± 0.017	0.1510 ± 0.008	1.289 ± 0.004	0.659 ± 0.008
320	0.366 ± 0.011	0.196 ± 0.011	0.267 ± 0.016	0.1390 ± 0.008	1.361 ± 0.004	0.696 ± 0.009

Note: Data given as mean ± standard deviation of triplicate test

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Many studies have suggested that the inhibitory mechanism of polyphenols against glycation is partly due to their antioxidant properties that hinder the autooxidation of amadori products.[33] Based on these data, it is reasonable that *C. hirta* extract had higher antiglycation activity than *M. affine* extract because it contained more phenolic compounds, flavonoids, and tannins, and also had higher antioxidant activity, as proven by the DPPH assay.

Methanol and ethyl acetate extracts of *C. hirta* leaves from Selangor, Malaysia were also reported to possess high SPF values (around 21) at a concentration of 500 ppm along with high DPPH antioxidant activity.[34] Considering the large production of herbal extracts for sunscreen products, the ethanol solvent in our study is relatively safer to handle and preferable to other organic solvents; therefore, our study provided evidence for the application of the ethanol extract of *C. hirta* leaves for sunscreen production. A previous study reported that *Tibouchina pulchra* Cogn., a Melastomataceae species, had different genetic structures in different altitudes (low and high).[35] Different environmental conditions, such as elevational gradients, may influence the genetic structure of plants, which may in turn affect its metabolite production. In this study, *C. hirta* growing in Mount Merapi National Park may have different genetic structures and metabolites compared to *C. hirta* growing in other areas because of the elevational gradient and other environmental conditions. Hence, this study displays the bioprospection of *C. hirta* which specifically grows in Mount Merapi National Park, for cosmeceutical use.

The results of this study suggested that ethanol leaf extracts of *C. hirta* from Mount Merapi National Park, Indonesia can potentially be used as a photo-protecting material by absorbing UV rays, scavenging ROS, and restraining the formation of AGEs in the skin. Other important photoaging-related mechanisms, such as anti-inflammatory effects, need to be investigated in vitro and in vivo to comprehensively understand the mechanism of the extract in antiphotoaging. Moreover, the application of *C. hirta* ethanol extract in sunscreen products requires optimization of the concentration and specific formulation to meet product claims.

Table 5: Antioxidant and antiglycation activity of extracts

Sample	IC_{50} (mg/L)	
	Antioxidation activity	Antiglycation activity
Clidemia hirta (L.) D.Don	2.55	161.23
Melastoma affine D.Don	4.71	251.47
Ascorbic acid	3.88	N.A.
Aminoguanidine	N.A.	22.34

N.A.: Not applicable

Conclusion

In conclusion, the ethanol extract of *C. hirta* leaves had higher SPF values, antioxidant and antiglycation activities than the ethanol extract of *M. affine* leaves. These bioactivities were possibly due to phenolic compounds, flavonoids, and tannins, which were higher in the ethanol extract of *C. hirta* leaves compared to that of *M. affine* leaves. The ethanol extract of *C. hirta* leaves was developed as a potential ingredient to be used in sunscreen products for skin photoprotection.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We would like to thank the Research and Community Service Department, Universitas Atma Jaya Yogyakarta, for providing financial support through University Internal Grant No.126/In-Pen/LPPM/VI/2021.

References

1. World Health Organization. Global solar UV index: a practical guide : a joint recommendation of World Health Organization, World Meteorological Organization, United Nations Environment Programme, International Commission on Non-Ionizing Radiation Protection. Geneva: WHO; 2002.
2. Valejo Coelho MM, Matos TR, Apetato M. The dark side of the light: mechanisms of photocarcinogenesis. Clin Dermatol. 2016;34(5):563–70.
3. Liu-Smith F, Jia J, Zheng Y. UV-induced molecular signaling differences in melanoma and non-melanoma skin cancer. In: Ahmad SI (Eds.). Ultraviolet light in human health, diseases and environment. Cham: Springer International Publishing; 2017; 27–40 p
4. Lee EJ, Kim JY, Oh SH. Advanced glycation end products (AGEs) promote melanogenesis through receptor for AGEs. Sci Rep. 2016 Jun 13;6(1):1–11.
5. Sell DR, Monnier VM. Molecular basis of arterial stiffening: role of glycation – A Mini-Review. GER. 2012;58(3):227–37.
6. Dale Wilson B, Moon S, Armstrong F. Comprehensive review of ultraviolet radiation and the current status on sunscreens. J Clin Aesthet Dermatol. 2012;5(9):18–23.
7. Latha MS, Martis J, Shobha V, Sham Shinde R, Bangera S, Krishnankutty B, Bellary S, Varughese S, Rao P, Naveen Kumar BR. Sunscreening agents. J Clin Aesthet Dermatol. 2013;6(1):16–26.
8. Radice M, Manfredini S, Ziosi P, Dissette V, Buso P, Fallacara A, Vertuani S. Herbal extracts, lichens and biomolecules as natural photo-protection alternatives to synthetic UV filters. A systematic review. Fitoterapia. 2016;114:144–162.
9. Saewan N, Jimtaisong A. Natural products as photoprotection. J Cosmet Dermatol. 2015;14(1):47–63.
10. Donglikar M, Deore S, Deore S, Deore S. Sunscreens: A review. Pharmacogn J. 2016;8(3):171–179.
11. de Lima Cherubim D, Buzanello Martins C, Oliveira Fariña L, da Silva de Lucca R. Polyphenols as natural antioxidants in cosmetics applications. J Cosmet Dermatol. 2020;19(1):33–37.
12. Ramadan A, Kamel G, Shokry A, El-Shiekh R. A potent anti-ageing and immunomodulatory activity of apricot seed standardized extract and its major compound; amygdalin. Trop J Nat Prod Res. 2020;4(10):728–733.
13. Nurwijayanto A, Na'iem M, Syahbudin A, Wahyuono S. Eksplorasi antioksidan tumbuhan obat yang berasal dari Taman Nasional Gunung Merapi Yogyakarta Indonesia. J Indones Med Plant. 2020;13(1):25–31.
14. Kementerian Kesehatan Republik Indonesia. Farmakope Herbal Indonesia Edisi II. Jakarta: Kementerian Kesehatan Republik Indonesia; 2017. 526–531 p.
15. Ahmed R, Tariq M, Hussain M, Andleeb A, Masoud MS, Ali I, et al. Phenolic contents-based assessment of therapeutic potential of

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Syzygium cumini leaves extract. PLOS ONE. 2019;14(8):e0221318.
16. Galvão MAM, Arruda AO de, Bezerra ICF, Ferreira MRA, Soares LAL. Evaluation of the Folin-Ciocalteu method and quantification of total tannins in stem barks and pods from *Libidibia ferrea* (Mart. ex Tul) L. P. Queiroz. Braz arch biol technol. 2018;61.
17. Tabasum S, Khare S, Jain K. Spectrophotometric quantification of total phenolic, flavonoid, and alkaloid contents of *Abrus precatorius* L. Seeds. Asian J Pharm Clin Res. 2016;9:371–374.
18. Wu X, Jia L, Wu J, Liu Y, Kang H, Liu X, Li P, He P, Tu Y, Li B. Simultaneous determination and quantification of triterpene saponins from *Camellia sinensis* seeds Using UPLC-PDA-QTOF-MS/MS. Molecules. 2019;24(20):3794.
19. Mansur JS, Breder MNR, Mansur MCA, Azulay RD. Determinação do fator de proteção solar por espectrofotometria. An Bras Dermatol Rio De Janeiro. 1986;61:121–124.
20. Ranjit R, Shrestha R, Paudel S, Maharjan J, Devkota B, Bhattarai S, Pandey B. Evaluation of biological properties and isolation of metabolites of Lichens of Parmeliaceae family from Himalayan Region of Nepal. Trop J Nat Prod Res. 2019;3(8):265–271.
21. Kim HY, Kim K. Protein glycation inhibitory and antioxidative activities of some plant extracts in vitro. J Agric Food Chem. 2003;51(6):1586–91.
22. Saewan N, Jimtaisong A. Photoprotection of natural flavonoids. J App Pharm Sci. 2013;3(9):129–41.
23. Figueiredo CCM, da Costa Gomes A, Granero FO, Bronzel Junior JL, Silva LP, Ximenes VF, da Silva RMG. Antiglycation and antioxidant activities of the crude extract and saponin fraction of *Tribulus terrestris* before and after microcapsule release. J Integr Med. 2022;20(2):153–62.
24. Silva RMG, Martins GR, Nucci LMB, Granero FO, Figueiredo CCM, Santiago PS, Silva LP. Antiglycation, antioxidant, antiacne, and photoprotective activities of crude extracts and triterpene saponin fraction of *Sapindus saponaria* L. fruits: An in vitro study. Asian Pac J Trop Biomed. 2022;12(9):391-399.
25. Ebrahimzadeh MA, Enayatifard R, Khalili M, Ghaffarloo M, Saeedi M, Yazdani Charati J. Correlation between sun protection factor and antioxidant activity, phenol and flavonoid contents of some medicinal plants. Iran J Pharm Res. 2014;13(3):1041–7.
26. Choquenet B, Couteau C, Paparis E, Coiffard LJM. Quercetin and rutin as potential sunscreen agents: determination of efficacy by an *in vitro* method. J Nat Prod. 2008;71(6):1117–8.
27. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol. 1988;124(6):869–871.
28. Arteaga JF, Ruiz-Montoya M, Palma A, Alonso-Garrido G, Pintado S, Rodríguez-Mellado JM. Comparison of the simple cyclic voltammetry (CV) and DPPH Assays for the determination of antioxidant capacity of active principles. Molecules. 2012;17(5):5126–38.
29. Omodamiro OD, Ajah O, Ewa-ibe C. Evaluation of antioxidant potential and anti-diabetic effect of ethanol seed extract of *Garcinia kola* (Bitter Kola) in albino rat. J Med Herbs Ethnomed. 2020;6:56–60.
30. Asgharpour Dil F, Ranjkesh Z, Goodarzi MT. A systematic review of antiglycation medicinal plants. Diabetes Metab Syndr. 2019;13(2):1225–9.
31. Bairagi J, Saikia PJ, Boro F, Hazarika A. A review on the ethnopharmacology, phytochemistry and pharmacology of *Polygonum hydropiper* Linn. J Pharm Pharmacol. 2022;74(5):619–645.
32. Kaewnarin K, Rakariyatham N. Antioxidant and antiglycation activities of some edible and medicinal plants. Chiang Mai J Sci. 2014;41(1):105–16.
33. Crascì L, Lauro MR, Puglisi G, Panico A. Natural antioxidant polyphenols on inflammation management: Anti-glycation activity vs metalloproteinases inhibition. Crit Rev Food Sci Nutr. 2018;58(6):893–904.
34. Azahar NF, Gani SSA, Hasanah U, Bawon P. Photo-protective and anti-oxidative potential in the leaves of three different Melastomataceae family species. J Mech Cont & Math Sci. 2019;(1):236–247.
35. Brito VLG, Sazima M. *Tibouchina pulchra* (Melastomataceae): reproductive biology of a tree species at two sites of an elevational gradient in the Atlantic rainforest in Brazil. Plant Syst Evol. 2012;298(7):1271–1279.

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License