ABSTRACT

The skin is the organ of the body that is frequently exposed to direct UV rays from the sun, which causes the formation of ROS (reactive oxygen species), leading to cell death and tissue damage. This health problem can be overcome by using antioxidants to stabilize the free radicals. Mustard as an organic substance which contains polysaccharide compounds, vitamin C, carotene, quercetin, routine compounds, kaempferol and β-cytostreol, all of these are beneficial to skin health and beauty. Therefore, this study aims to determine the antioxidant activity of the total flavonoids and phenol content of mustard ethanol extract using the DPPH method. Furthermore, the extract at concentrations of 2, 4, and 6% was also tested for its anti-aging activity using the following parameters: moisture and oil content, texture, collagen, wrinkles, pigment, sensitivity, and pores. The results showed that the antioxidant activity of the green mustard had an IC₅₀ value of 170.7839 µg/ml, a total phenol content of 14.471 mg GAE/g extract and flavonoid content of 12.753 mg QE/g extract. The effectiveness of the aging activity of the extract was tested using formulation 6%, which is better than 2%, and 4%. Thus, the percentage of water content recovery, oil content, texture, collagen, wrinkles, stains, sensitivity and pores

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respectively was 36.41, 39.33, 64.07, 17.65, 65.28, 50.14, 49.10 and 35.09%. Consequently, it was concluded that the extract has the potential of being developed into a herbal beauty product.

Keywords: Anti-aging; antioxidant; green mustard.

1. INTRODUCTION

The skin is the organ of the body that is frequently exposed to direct UV rays from the sun which then initiate the formation of ROS (reactive oxygen species) [1]. Furthermore, free radicals caused by ROS damages the cells and tissues which eventually leads to aging and cell death. Visible symptoms of skin aging include wrinkled dry rough skin, the appearance of black spots, and enlarged pores [2,3,4]. However, this health problem can be fixed by using antioxidants to stabilize free radicals [5].

Mustard greens could become a source of these antioxidants as they contain vitamin A and C, and flavonoids which act as antioxidants [6,7]. Furthermore, mustard greens also contains polysaccharide compounds, carotene, quercetin, routine compounds, kaempferol, and beta-sitosterol all of which have benefits for skin health and beauty. Due to its rich content, these greens could make the skin become smooth, moisturized, bright and also prevent various skin health problems [7].

To facilitate its use and speed up the delivery of antioxidants, mustard greens should be formulated in gel form. The gel possesses good dispersibility and absorption, does not inhibit physiological functions, and keeps pores unclogged. Furthermore, it also possess other advantages such as providing a cool sensation when applied, being easy to wash and apply, acting as a good drug release system, and is stable under storage [8,9].

2. METHODS

The methodology used in this research is an experimental laboratory method, including sample preparation, phytochemical screening, extract making, antioxidant testing using the DPPH method, gel formulation, irritation test on 12 volunteers and anti-aging effectiveness test.

2.1 Materials

Green mustard, ethanol 96%, DPPH, 2 N HCl, concentrated $\text{H}_2\text{SO}_4$, HPMC, n-hexane, methanol, isopropanol, amyl alcohol, 1% FeCl$_3$, 0.4 M lead (II) acetate, Meyer's reagent, Bouchardat's reagent, Dragendorff's reagent, reagent Molish, Liebermann-Burchard reagent, Mg powder, aquadest, propylene glycol, glycerin, nipagin, triethanolamine.

2.2 Instrument

Laboratory glassware, blender, filter paper, drying cabinet, porcelain mortar, analytical balance (Dickson), rotary evaporator, UV-Vis spectrophotometer (Shimadzu), skin analyzer (Skin observed system).

2.3 Plant Collection and Identification

Green mustard was taken purposively without comparing with the same plants from other areas. The plants were taken from the Medan District, PancurBatu. Identification of mustard material was carried out at the Herbarium Medanense (MEDA), Faculty of Mathematics and Natural Sciences, University of North Sumatra, Medan.

2.4 Extraction

Green mustard was prepared in stages such as: washing, wet sorting, drying, dry sorting, and storage. It was then macerated with 96% ethanol for 7 days at room temperature, in a closed container protected from sunlight [10].

2.5 Phytochemical Screening

This test was carried out in accordance with the Indonesian Herbal Pharmacopeia, by the qualitative identification of compounds such as alkaloids, flavonoids, tannins, steroids, saponins, glycosides [10].

2.6 Antioxidant Activity Test

Antioxidant activity testing was carried out using the free radical scavenging method (DPPH) (1,1-diphenyl-2-picrylhydrazyl). Furthermore, 2 ml of DPPH solution (200 ppm in methanol) was mixed with 0, 0.4, 1.2, and 2 ml of green mustard
ethanol extract (green mustard ethanol extract 500 ppm) to obtain concentrations of 0.20, 40, 60, 80, 100 ppm. Each solution was shaken and incubated in the dark at room temperature for 30 minutes and the absorbance was measured at a wavelength of 516 nm. In addition, vitamin C was used as a means of comparison. The inhibition of the samples DPPH free radicals was calculated based on the formula below [11]:

\[
\text{DPPH Trapping Activity (\%) = } \frac{\text{abs blanko (DPPH)} - \text{abs sample}}{\text{abs blanko (DPPH)}} \times 100
\]

2.7 Determination of Total Phenol Content

The total phenol content was calculated using the spectrophotometric method. Furthermore, 0.1 mL of the sample (1000 ppm) was mixed with 0.5 mL of folin-ciocalteu reagent, 7.9 mL of distilled water, and stirred for ±1 minute, after which 1.5 mL of 20% Na₂CO₃ was added, and then incubated for 90 minutes. Total phenol was determined from a calibration curve of gallic acid with a concentration of 0; 31.25; 62.5; 125; 250 ppm. Consequently, the determination of the calibration curve was carried out using the same method. Finally, the absorbance was measured at a wavelength of 775 nm [12].

2.8 Determination of Total Flavanoid Content

Total flavonoid levels were calculated using the spectrophotometric method, which entailed 2 mL of the sample (1000 ppm) being mixed with 0.1 AlCl₃, 0.1 mL CH₃COONA, and 2.8 mL of distilled water vortex for ±1 minute, and then incubated for 40 minutes. Total flavonoid was determined using a quercetin calibration curve with a concentration of 0; 6; 14.5; 19; 23.5 ppm. The determination was carried out using the same method, after which the absorbance was measured at a wavelength of 440 nm [13].

2.9 Formulation of Ointments

Stages of manufacture HPMC was manufactured in hot water (temperature 70°C) and allowed to develop completely. Subsequently, propylene glycol, glycerin, and methylparaben solution were mixed in hot distilled water and crushed until homogeneous, after which triethanolamine was added to form a gel [14]. Green mustard ethanol extract was gradually added to the base of the gel during grinding until a homogeneous mixture was obtained.

2.10 Irritation Test for Volunteers

The gel was applied with a diameter of ±3 cm to the forearm of volunteers, then left for 24 hours for the observation of any redness, itching and crusting [4].

2.11 Anti-aging Effectiveness Testing

Samples were obtained (using a simple random sampling method) from affordable populations that met the inclusion and exclusion criteria, such as being double blind. Furthermore, 4 formulas F0, F1, F2, F3 (0; 2; 4; 6% green mustard ethanol extract) were analyzed by pretest-and posttest designs using parameters such as water and oil content, texture, collagen, wrinkles, pigment, sensitivity, and pores. These parameters were checked using using a skin analyzer.

| Material          | Concentration (%) |
|-------------------|-------------------|
|                   | F0    | F1    | F2    | F3    |
| Green Mustard     | -     | 2     | 4     | 6     |
| extract           |       |       |       |       |
| HPMC              | 1     | 1     | 1     | 1     |
| Propylene glycol  | 10    | 10    | 10    | 10    |
| Glycerin          | 5     | 5     | 5     | 5     |
| Nipagin           | 0,1   | 0,1   | 0,1   | 0,1   |
| Triethanolamine   | q.s   | q.s   | q.s   | q.s   |
| Aquadest          | 100   | 100   | 100   | 100   |

Table 1. Topical dosage formulations for each gel
2.11.1 Inclusion criteria
1. Able-bodied male and female
2. Productive age (20-25 years)
3. No history of any allergy-related diseases
4. Should be willing to receive treatment using the gel, by applying it 2x a day (morning-night) for 4 weeks.

2.11.2 Exclusion criteria
1. Irritation to the gel preparation
2. Having a history of diseases related to allergies
3. Already using treatments from a dermatologist

The measurement results are described in Table 2.

2.12 Statistical Analysis
The research data was analyzed using the SPSS (Statistical Product and Service Solution) program 21.

Table 2. The parameters of the anti-aging effectiveness test

| Measurement | Parameter |
|-------------|-----------|
| (Moisture)  | Extremely Dry | Less Dry | Dermis Dry | Epidermis Dry | Moist |
|             | 0-11       | 11-37    | 37-56     | 56-75        | 75-100 |
| (Oily)      | Less       | Balance  | Oily      | Over         | Oily Pustule |
|             | 0-13       | 13-37    | 37-39     | 79-91        | 91-100 |
| (Texture)   | Perfect    | Little Rough | Rough | Irregular | Abnormal |
|             | 0-12       | 12-24    | 24-36     | 36-66        | 66-100 |
| (Collagen)  | Split Up   | Loose Fiber | Seriously | Little Lost | Enough |
|             | 0-12       | 12-26    | 26-43     | 43-78        | 78-100 |
| (Wrinkle)   | Nothing    | Little Wrinkles | Irregular | Seriously |
|             | 0-12       | 12-24    | 24-26     | 36-66        | 66-100 |
| (Spot)      | Normal     | Spotted Freckles | Spots On The Epidermis | Seriously |
|             | 0-16       | 16-37    | 37-68     | 68-90        | 90-100 |
| (Sensitivity)| Normal Sensitivity | Little Reddish | Red Spots | Red Stain |
|             | 0-16       | 16-37    | 37-68     | 68-90        | 90-100 |
| (Pore)      | Normal Little Closed Pores | Blackheads | Seriously |
|             | 0-8        | 8-16     | 16-27     | 27-56        | 56-100 |

2.11.1 Inclusion criteria
3. RESULTS

3.1 Phytochemical Screening
The analysis was carried out to determine the content of secondary metabolites in mustard greens. Consequently, the results showed that green mustard contains alkaloids, glycosides, steroids/triterpenoids, flavonoids, tannins, and saponins. Then, further examination was carried out regarding the levels of phenols and flavonoids in the green mustard extract.

The total phenol content was measured using a colorimetric method which is based on the principle of the green mustard ethanol extract reduction capacity and the equivalent reduction of gallic acid. Furthermore, flavonoids contain a hydroxyl group that plays a role in antioxidant activity. An increase in free hydroxyl groups that donate hydrogen will also cause further reduction in free radicals. From the result it is inferred that phenolic and flavonoid compounds have a linear contribution to antioxidant activity. Therefore, the higher contents, better the antioxidants [15].

Table 3. Total of phenols and flavonoids content of green mustard extract

| Contents                  | Concentration | Level  |
|---------------------------|---------------|--------|
| Total Phenol (GAE/g Extract) | 14,586        | 14,471 |
| Total Flavonoid (QE/g Extract) | 12,855        | 12,753 |
Table 4. The results of anti-aging effectiveness test

| Parameter     | Testing Time | Treatment (Mean±SD) | F0          | F1          | F2          | F3          | P (Kruskal-Wallis) |
|---------------|--------------|---------------------|-------------|-------------|-------------|-------------|--------------------|
| Moisture      | Before       | 74.67±1.53          | 73.00±0.00  | 68.33±2.08  | 33.67±1.53  | 30.67±1.50  | 0.017              |
|               | After        | 89.67±1.52          | 94.33±1.15  | 94.00±5.29  | 53.00±2.65  | 50.00±4.93  | 0.048              |
| Oil Content   | Before       | 59.67±2.88          | 65.67±1.53  | 60.00±5.19  | 71.00±10.58 | 68.00±1.50 | 0.115              |
|               | After        | 46.33±3.51          | 49.33±4.04  | 37.33±3.05  | 43.00±6.55  | 40.00±1.00 | 0.100              |
| Texture       | Before       | 9.33±1.53           | 7.67±1.15   | 7.67±0.57   | 9.33±0.57   | 9.33±0.57   | 0.120              |
|               | After        | 4.33±0.57           | 3.00±0.00   | 2.67±0.57   | 3.33±0.57   | 3.33±0.57   | 0.044              |
| Collagen      | Before       | 86.67±2.31          | 81.33±1.53  | 80.33±2.31  | 79.33±0.57  | 79.33±0.57  | 0.044              |
|               | After        | 94.67±0.21          | 93.00±2.65  | 94.00±2.65  | 96.33±0.57  | 96.33±0.57  | 0.336              |
| Wrinkles      | Before       | 8.00±1.00           | 8.33±1.15   | 8.00±1.00   | 8.67±0.57   | 8.67±0.57   | 0.748              |
|               | After        | 5.00±1.00           | 4.67±0.57   | 3.67±0.57   | 3.00±0.00   | 3.00±0.00   | 0.044              |
| Spots         | Before       | 26.33±0.57          | 28.00±4.35  | 26.00±2.65  | 32.67±4.93  | 32.67±4.93  | 0.198              |
|               | After        | 21.33±0.57          | 21.00±3.46  | 16.67±2.08  | 16.33±2.88  | 16.33±2.88  | 0.050              |
| Sensitivity   | Before       | 20.67±1.15          | 22.67±1.53  | 22.67±1.53  | 26.33±3.78  | 26.33±3.78  | 0.150              |
|               | After        | 15.67±1.15          | 15.33±1.15  | 13.00±1.00  | 13.33±1.53  | 13.33±1.53  | 0.092              |
| Pores         | Before       | 1.00±0.00           | 1.00±0.00   | 9.33±2.08   | 34.33±38.73 | 34.33±38.73 | 0.018              |
|               | After        | 1.00±0.00           | 1.00±0.00   | 6.67±1.52   | 22±24.33    | 22±24.33    | 0.020              |

3.2 Antioxidant Test Using the DPPH Method

Antioxidant activity was assessed based on the IC50 value. The IC50 value of the mustard green ethanol extract was 170.7839 µg / ml (regression equation y = 0.2926x + 0.0190), while that of vitamin C was 2.693 g / ml (regression equation y = 17.548x + 2.7315). Green mustard ethanol extract has a weak antioxidant activity (IC50 value is 151-200 µg / ml), while that of vitamin C is very strong (IC50 value is less than 50 µg / ml) [11].

3.3 Safety of Green Mustard Ethanol Extract Gel

The test results showed that there was no irritation reaction such as redness, itching, and skin roughness in all volunteers. Therefore, the mustard green ethanol extract gel was concluded to be safe to use [2].

3.4 Anti-Aging Effectiveness

Tests were carried out using a skin analyzer system with parameters such as moisture and oil content, texture, collagen, wrinkles, pigment, sensitivity, and pores. The use of green mustard ethanol extract gel has an effect on facial skin in all test parameters and the best results were obtained with F3 gel (6% green mustard ethanol extract). Statistically, there was a significant difference before and after the 4 weeks of use on the parameters of the moisture, texture, collagen, wrinkles, and pores test. Meanwhile, there was no significant difference in the parameters for oil content, spot, and sensitivity test during the 4 weeks of use, although, there was an increase in skin recovery based on weekly tests.

4. DISCUSSION

The result of this study indicates that ethanol extract of green mustard contains alkaloids, glycosides, steroids / triterpenoids, flavonoids, tannins and saponins which have antioxidant activity. Then the green mustard ethanol extract contains flavonoids up to 12.753 mg QE / g extract, while the total phenol content in the gel is 14.471 mg GAE / g extract. Antioxidants are compounds that can neutralize free radicals which cause aging [16]. Consequently, flavonoids have a hydroxyl group that is an antioxidant [15].

Aging may be a physiological process that can’t be avoided; however, it can happen faster than it should, this phenomenon is named premature aging. At the age of 20-30 years, normal regeneration of skin cells takes place every 28-30 days, but this rate slows down as the person ages. When a person reaches 50 years old, skin regeneration takes place every 37 days [3]. The clinical manifestations of aging are dry and thin skin due to its reduced ability to form new cells; rough, dull, and scaly skin due to reduced skin cell regeneration; loose and inelastic skin due to its decreased ability to regenerate collagen,
which causes wrinkles and sagging; spotty skin color due to reduced pigmentation and distribution of melanocytes to all layers of the skin [4].

The skin becomes dry due to a reduction in the activity of the oil and sweat glands also its ability to retain water (skin barrier) [4]. Green mustard ethanol extract flavonoids moisturize the skin, therefore the extract concentration is linear with the properties of moisturizing facial skin [17]. Oily skin is caused by very productive oil glands in the dermis layer, however the antioxidant compound in green mustard ethanol extract cleanses the skin and regulates oil production [17]. The increased free radicals caused by UV exposure can cause cell/tissue damage to the skin's supporting tissue therefore reducing collagen fibers to MMP enzymes. Furthermore, UV radiation also affects the tyrosinase enzyme which plays a role in melanin synthesis, tyrosine hydroxylation, L-DOPA oxidation (3,4- dihydroxy-phenylalanine) and hydroxyindole oxidation. This enzyme works to convert tyrosine to 3,4-dihydroxy-phenylalanine (DOPA) and then to dopaquinone which goes through several stages of transformation to be converted into melanin. Pigmentation patches that are uneven on the skin's surface are due to changes in the distribution of melanin pigments accompanied by decreased melanocyte function [4]. Furthermore, sunlight causes skin cells to die and slows down their regeneration, thereby enlarging facial pores [17].

The success of a dermatological drug depends on its ability to penetrate the skin in sufficient quantities to achieve the desired therapeutic effect. Since consumers prefer to use more natural compounds on their skin, plant phenolic compounds are a promising target for new dermal cosmetics that possess the ability to maintain the skin homogeneity and a proper, healthy look due to effective skin cell renewal, elastin and collagen stimulation and inhibition of excessive melanin synthesis. The gel used in this study contains high levels of phenolic compound that has antioxidant activity. The antioxidant activity of phenolics is feasible through various mechanisms of action: inhibition of the ROS formation and therefore the ROS trapping and the extinction of singlet oxygen; and reducing the chelated metal ions (which are the catalysts for reactions resulting in the formation of ROS), interrupting the cascade of radical reactions in lipid peroxidation and protecting the opposite compounds with antioxidant activity. It was reported that a lot of polyphenols from plants exhibit inhibitory activity against collagenases and elastases, thus facilitating maintenance of proper skin structure. Presents the phenolic compounds involved within the preservation of the proper skin structure through the regulation of MMPs. Tests performed on skin cell cultures have shown that phenolic compounds are effective within the suppression of melanin synthesis. He melanin inhibitors are supposed to act mostly by the suppression of tyrosinase [133,134,135]. Phenolics and flavonoids, thanks to aromatic purine rings in their composition, have similar structures to tyrosine, which are oxidized by tyrosinase, and thus, they will act as substrate analog inhibitors against melanogenesis [18].

The antioxidant content of green mustard ethanol extract can reduce fine lines and increase collagen production thereby improving facial skin texture and maintaining natural skin beauty. Antioxidants (both phenolic and flavonoid compounds) are needed to regenerate and provide skin tissue structure [19]. They also stimulate the formation and production of collagen which maintains skin elasticity, flexibility, smoothness and reduce pore size [20,21]. Green mustard ethanol extract flavonoids directly inhibit tyrosinase activity in the melanogenesis process which prevents melanin from accumulating in the skin and causing blemishes [22]. Antioxidant compounds are also able to protect healthy skin from overreactions, irritations and allergies that can interfere with its health [23]. Therefore, the development of a gel based on hydroxyl-propyl-methyl-cellulose (HPMC), can stimulate the growth of skin cells by stimulating cell division and maintaining the tissue repair phase, as well as accelerating skin regeneration, and stimulating collagen formation [24,25].

5. CONCLUSION

In conclusion, green mustard has the potential to act as an anti-aging agent due to its moderate antioxidant activity (IC50 170.7839 μg/ml), as well as its phenol and flavonoid content (14.471 mg GAE / g extract and 12.753 mg QE / g extract). The gel created with the F3 formulation had the best anti-aging activity, the best percentage of moisture recovery (36.41%) and other parameters such as oil content, texture (64.07%), collagen percentage (17.65%), wrinkles (65.28%), spots (50.14%) sensitivity (49.10%) and pores (35.09%).
DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments have been approved by Health Research Ethics Committee from Universitas Prima Indonesia with registration no. 016/KEPK/UNPRI/I/2020.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/77359