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Research article

Sunscreen activities from ethanol extract of *Wodyetia Bifurcata* L (family Arecaceae) fruit

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ABSTRACT

This study aimed to determine the sunscreen activity of ethanol extract, subfraction, pure isolate and lotion of *Wodyetia bifurcata* fruit. In addition, the physical and organoleptic properties of the extract were determined. Sunscreen activity was measured using UV spectrophotometry, and sun protection factor (SPF) values were used. The results show that at the same concentration (extract 12%), the highest SPF value is 39.144±0.450 and the lotion is 37.039±0.368; the lowest SPF value is 36.815±0.067, and the lotion is 25.798 ± 0.392 (subfraction B). The SPF values of the pure isolate and lotion are 9.104±0.268 and 6.091±0.187, respectively. The physical properties of the extract lotion comply with the 1996 Indonesian national standards, and the most preferred formula is 2%. Gas chromatography-mass spectrometry characterization results showed that the main compound in the ethanol extract was oleic acid (18.39%). In comparison, the pure isolate obtained was *β*-sitosterol (4.56%), which was confirmed by proton nuclear magnetic resonance (NMR) and carbon-13 NMR data. Extract, subfraction, and pure isolate of *W. bifurcata* fruit have potential as sunscreen agents based on SPF values.

Keywords: *Wodyetia bifurcata*, sunscreen, SPF, lotion, *β*-sitosterol.

INTRODUCTION

Our skin serves as the first line of defense against various types of harm, ranging from extreme heat to chemical exposure and even UV rays [1]. However, over exposure to UV radiation can lead to negative outcomes, such as sunburn, skin discoloration, and even cancer. This is due to the formation of reactive oxygen species (ROS) resulting from UV-A and UV-B radiation, which can heighten oxidative stress on vital skin components and lead to cellular dysfunction [2].

Sunscreen is one product that can reduce the UV radiation energy on the skin's surface; therefore, it has been widely used to protect the skin from sun exposure. Sunscreen is a practical step to avoid skin problems caused by sunlight [3].

The effectiveness of sunscreen can be determined based on the value of the Sun Protection Factor (SPF), which is defined as the minimum time for the appearance of erythema on the skin after UV irradiation [4]. The SPF value is influenced by the active compounds that act as antioxidants.

Synthetic antioxidants such as Butyl Hydroxy Anisol (BHA) and Butyl Hydroxy Toluene (BHT) in the long term can cause irritation, burning, contact dermatitis, damage, and cancer on the skin [5]. Natural antioxidants can also be used as active sunscreen compounds that potentially eliminate free radicals [6]. One plant with antioxidant activity is *W. bifurcata*, which contains flavonoids, phenolics, steroids, and triterpenoids. The compounds contained in the *W. bifurcata* plant

include *β*-amyrin and lupeol, which are triterpenoid compounds; apigenin and kaempferol, which are flavonoid compounds; 4-hydroxybenzoic acid and gallic acid, which are phenolic compounds [7]. Therefore, this study aimed to determine the sunscreen activity of the ethanol extract, subfraction, and pure isolate of the *W. bifurcata* fruit.

MATERIALS AND METHODS

Sample Collection and Extraction Method

The chemicals used in this study were stearic acid (Merck 57-11-4), Cetyl alcohol (Merck 36653-84-3), lanolin (Merck 8006-54-0), Triethanolamin (Merck 102-71-6), glycerin (Merck 56-81-5), KLT60 F_{254} plate, distilled water, methyl paraben (Merck 99-76- 3), silica gel 60 (Merck 7631-86-9), ethanol (Merck 64-17-5), *n*-hexane (Merck 110-54-3), chloroform (Merck 67-66-3), ethyl acetate (Merck 141-78-6), Mg metal (Merck 7439-95-4), HCl (Merck 7647-01-0), ammonia (Merck 7664-41-7), reagents for phytochemicals analysis (Liebermann-Burchard, Dragendroff's, Mayer and Wagner were purchased from Sigma-Aldrich), Folin Ciocalteu regent (Sigma-Aldrich), quercetin (Sigma-Aldrich), aluminum chloride (Merck), sodium carbonate (Merck), potassium acetate (Merck), DPPH (Sigma-Aldrich), and methanol (Merck).

Plant Collection

W. bifurcata fruits were collected from Tapaktuan-Meulaboh National road, Kuta Baro, Meukek district, South Aceh in Aceh Province (3.456805, 97.059604). Dr. Saida Rasnovi identified the sample, number 13/UN11.1.8.4/TA.00.03/2023, at the Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. The methodology used in phytochemical testing can be found in phytochemical methods, a guide to modern techniques of plant analysis [8].

Extraction of *W. bifurcata* Fruit

The fruits of *W. bifurcata* were cleaned and split into two parts, and the fruit was then separated between the mesocarp and kernel. The mesocarp of the fruit was collected, dried, and mashed. Dry powder of *W. bifurcata* fruits was weighed as much as 3 kg, then macerated using *n*-hexane for 3 x 24 hours at room temperature. The resulting extract was evaporated using a rotary evaporator. This procedure was repeated until a clear extract was obtained. The *n*-hexane extract obtained as much as 19.151 g (0.63%). The residue was macerated again using ethanol 96%, with the same treatment as *n*-hexane. The ethanol extract obtained as much as 192.731 g (6.424%). Both extracts were tested for phytochemical and sunscreen activities. In this study, we compared the SPF values of each extract and commercial sunscreen (SPF 30). The test results showed that the ethanol extract had a higher sunscreen activity than the *n*-hexane extract; therefore, the ethanol extract was characterized by GC-MS (Shimadzu QP 2010 Ultra), followed by fractionation and isolation. In addition, the ethanol extract, subfractions, and pure isolate were prepared in lotion form, and their SPF values were tested. The ethanol extract lotion's physical properties (emulsion type, viscosity, pH, Spreadability, and adhesion) and organoleptic properties (color, fragrance, and texture) were then tested for both extracts and the isolated compound.

Fractionation of Ethanol Extract

About 30 g of extract ethanol was subjected into the column chromatography process. The compound components were separated by the elution gradient method. The sample in the column containing silica gel 60 was eluted with 100% *n*-hexane, followed by the ratio of *n*-hexane: ethyl acetate (9:1) to (1:9), and 100% ethyl acetate. The separation process produced 94 fractions with 3 subfraction groups. The subfractions were A (1.1093 g), B (0.3181 g), and C (0.4306 g), respectively. The results of the sunscreen activity test showed that subfraction A had a high SPF value and the highest amount; therefore, re-chromatography was performed on this subfraction.

The subfraction A was re-chromatographed with 100% *n*-hexane eluent and continued with *n*-hexane: ethyl acetate (9.5:0.5) until a pure isolate stain pattern was obtained. The resulting pure isolate was tested using three different eluent ratios: *n*-hexane: chloroform (8:2), *n*-hexane: ethyl acetate (5:5), and *n*-hexane: ethyl acetate (8:2). The pure isolate was characterized by ^{1}H-NMR and ^{13}C-NMR (Agilent 500 MHz DDR2 Waters LCT Premier XE).

Making Sunscreen Lotion

The preparation of the sunscreen lotion for *W. bifurcata* was based on previous studies [6, 9], with modifications as necessary. Table 1 presents the basic formulations of *W. bifurcata* fruit ethanol extract lotion at various concentrations.

Table 1: The Formula of lotion preparation (with modification)

Number	Material	Composition (%)				
I (Oil phase)	Cetyl alcohol	0.5	0.5	0.5	0.5	0.5
	Stearic acid	3	3	3	3	3
	Lanolin	1	1	1	1	1
II Water phase)	Glycerin	2	2	2	2	2
	Methyl paraben	0.1	0.1	0.1	0.1	0.1
	Triethanolamin	0.7	0.7	0.7	0.7	0.75
	Aquades	90.	90.	87.	85.	80.65
III	*W. bifurcata*	2	2.5	5	7.5	12

All required ingredients were weighed according to a predetermined composition. The materials in the oil phase are placed in a porcelain cup and heated to 70°C. The materials in the water phase were dissolved in a beaker. The Water phase was added to the hot oil phase and stirred until the temperature decreased. *W. bifurcata* ethanol extract was added when the temperature dropped to 45°C [6].

Measurement of the Sun Protection Factor (SPF) Value

The method for the absorption of sunscreen agents is determined based on spectrophotometric analysis [10]. The lotion sample was measured at 20.000 ppm, that is, the sample (0.5 g) was dissolved in 25 mL ethanol [6]. The absorbance of the sample was measured using a UV spectrophotometer (1240 Shimadzu UV-Vis mini), every 5 nm with a wavelength range of 290 nm-320 nm.

Measurement of the SPF value is determined using the equation 1 [11].

$$SPF = CF \sum_{290}^{320} EE(\lambda)\, I(\lambda)\, A(\lambda) \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Where: EE (λ) is the spectrum of the erithermal effect; I (λ) is solar intensity spectrum; Abs (λ) is the absorbance of the sunscreen product; CF is correction factor (=10) and the value of EE × I are constants [10].

Testing the Physical Properties of the Lotion

Emulsion Type Determination

The lotion preparation's emulsion type was tested using an ammeter. The ammeter will produce a current if the preparation has an o/w emulsion (oil in water) [12].

Viscosity Determination

The sample was placed in a beaker, and the viscosity was measured using a viscometer mounted on a spindle. The lotion viscosity was measured using a Thermo Scientific HAAKE Viscometer C Viscometer [6].

pH Value Determination

The sample was diluted with distilled water and placed in a beaker (lotion and distilled water at a ratio of 1:9), and the pH meter electrode was dipped into the prepared sample [6].

Spreadability Determination

A lotion (0.5 g) was placed on a glass slide. Another round of glass was placed on top of the lotion, and a load of 100 g was placed on the glass for 1 min. Spread power was measured using a ruler [6].

Adhesion Determination

A lotion (5 g) was placed on a round glass slide. Another round of glass was placed on top of the lotion, and a load of 500 g was placed on the glass for 5 min. The load and glass above the lotion were removed using a load of 80 g, and the time the glass was released was calculated [13].

Organoleptic Tests

Organoleptic tests include sensory examination of colour, fragrance, and texture. The test was carried out on each extract lotion, namely at concentrations of 2%, 2.5%, 5%, 7.5%, and 12%, to 30 panellists who are sunscreen consumers in Universitas Syiah Kuala. The panellists selected the preference level for each variation in the most preferred concentration and lotion. The level of preference was determined by measuring 1-5 (1: very like, 2: like, 3: little like, 4: dislike, and 5: immensely dislike) [14].

Determination of Total Phenolic Content

Gallic acid (10 mg) was added to distilled water in a 10 mL volumetric flask up to the mark (1000 ppm). The stock solution of 1000 ppm gallic acid, then created in a series of concentrations are 100 ppm, 125 ppm, 150 ppm, 175 ppm, and 200 ppm. Each concentration of gallic acid was taken at a much of 0.2 mL, and then 0.2 mL gallic acid solution was added to 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent. The solution was shaken until homogeneous and left for 5 min. Furthermore, 3 mL of 10% Na_2CO_3 was added to the solution and incubated for 90 min at room temperature. The absorbance of each solution was measured at a maximum wavelength of 765 nm using a UV-Vis spectrophotometer [15] with some modifications. A calibration curve was constructed by plotting the concentration of gallic acid against absorbance to obtain the regression equation.

Sample treatment (1000 ppm) was performed according to the standard solution. The absorbance of the sample solution was measured at a maximum wavelength of 765 nm using a UV-Vis spectrophotometer [15] with some modifications.

Determination of Total Flavonoid Content

Quercetin (10 mg) was added to methanol p.a in a 10 mL volumetric flask up to the mark (1000 ppm). The solution was taken at a much of 1 mL, and then the volume was added to 10 mL with methanol p.a (100 ppm). The stock solution of 100 ppm quercetin standard then created a series of concentrations are 2, 4, 6, 8, and 10 ppm. Each concentration of quercetin was taken at a much of 2 mL, and then 2 mL quercetin solution was added to 6 mL methanol, 0.4 mL $AlCl_3$, and 0.4 mL KCH_3COO 1 M, and 11.2 mL Aquades. The solution was incubated at room temperature for 30 min, and the absorbance of each solution was measured at a wavelength of 440 nm using a UV-Vis spectrophotometer [16] with some modifications. A calibration curve was constructed by plotting the concentration of quercetin against absorbance to obtain the regression equation.

Sample treatment (1000 ppm) was performed according to the standard solution. The absorbance of the sample solution was measured at a maximum wavelength of 440 nm using a UV-Vis spectrophotometer [16] with some modifications.

Determination of Antioxidant Activity

Two mL of 0.5 mM DPPH solution (200 ppm) was added to a 10 mL volumetric flask and diluted with methanol to obtain a solution with a concentration of 40 ppm. The DPPH solution was measured in the 400-800 nm wavelength range to obtain the maximum wavelength used for the sample and quercetin measurements [17].

Ten mg of *W. bifurcata* fruit extract and ascorbic acid were dissolved in methanol in a 10 mL volumetric flask (1000 ppm). 1000 ppm extract solution was taken at a much of 0.01 mL, 0.05 mL, 0.1 mL, 0.5 mL, and 1 mL into a 10 mL volumetric flask to obtain concentrations of 1 ppm, 5 ppm, 10 ppm, 50 ppm, and 100 ppm. 1000 ppm ascorbic acid was taken at a much of 0.02 mL, 0.03 mL, 0.04 mL, and 0.05 mL into a 10 mL volumetric flask to obtain concentrations of 2 ppm, 3 ppm, 4 ppm, and 5 ppm as a positive control. Two mL of DPPH solution and methanol were added to each volumetric flask up to the mark. The solution was homogenized and incubated for 15 min. The solution was measured at a maximum wavelength of 516 nm using a spectrophotometer UV-Vis [17] with some modifications.

RESULTS AND DISCUSSION

Phytochemicals of *W. bifurcata* Fruit

The *n*-hexane and ethanol extracts contain terpenoid and steroid secondary metabolites. Saponins, flavonoids, and phenolics

were found only in ethanol extract. The appearance of a red color indicated the presence of terpenoids in both extracts, and steroids were indicated by a brownish-green color when reacted with Liebermann-Burchard. The color change occurs because of the reaction of acetic anhydride with acids to form a carbocation in the anhydride, which binds to the O atom in the OH group of terpenoid or steroid compounds [18].

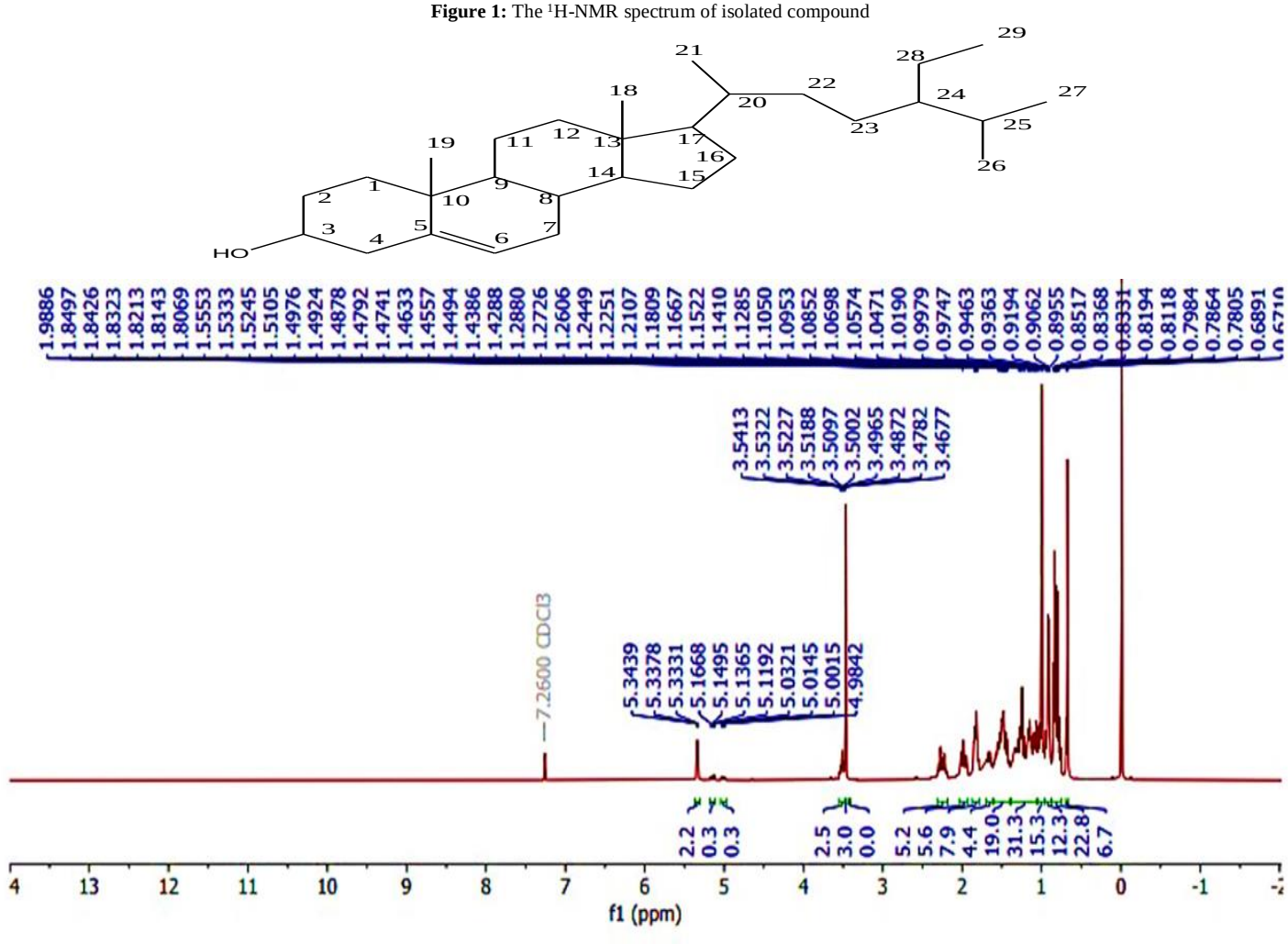
The phenolic compounds were characterized by the emergence of a dark green color caused by the formation of a complex of Fe^{3+} ions with phenol groups [19].

Flavonoid compounds were indicated by the formation of a purple color. The color change occurred because of the presence of Mg and HCl, which reduced the benzopyrone nucleus so that flavilium salts were formed [19]. Saponin compounds were characterized by the appearance of foam formed after the addition of water owing to the presence of hydrophilic and hydrophobic groups [20]

Analysis of Isolated Compound Using ^{1}H-NMR and ^{13}C-NMR

The ^{1}H-NMR spectrum of the isolated compound was identical to reported β-sitosterol compound [21]. This characteristic signal was found in the positions of protons H-18 (δ_H 0.67 ppm) and H-19 (δ_H 0.99 ppm), which exhibited two singlet proton signals. The ^{1}H-NMR spectrum of the isolated compound is shown in Figure 1.

Figure 1: The ^{1}H-NMR spectrum of isolated compound



The positions of the protons H-21, H-27, H-26, and H-29 resonated as doublet peaks at δ_H 0.78; 0.81; 0.83; and 0.90 ppm, respectively. The ^{1}H-NMR spectrum is similar to the reported β-sitosterol spectrum [21]. A comparison of the ^{1}H-NMR and ^{13}C-NMR spectral data of the isolated compound with the β-sitosterol compound is presented in Table 2.

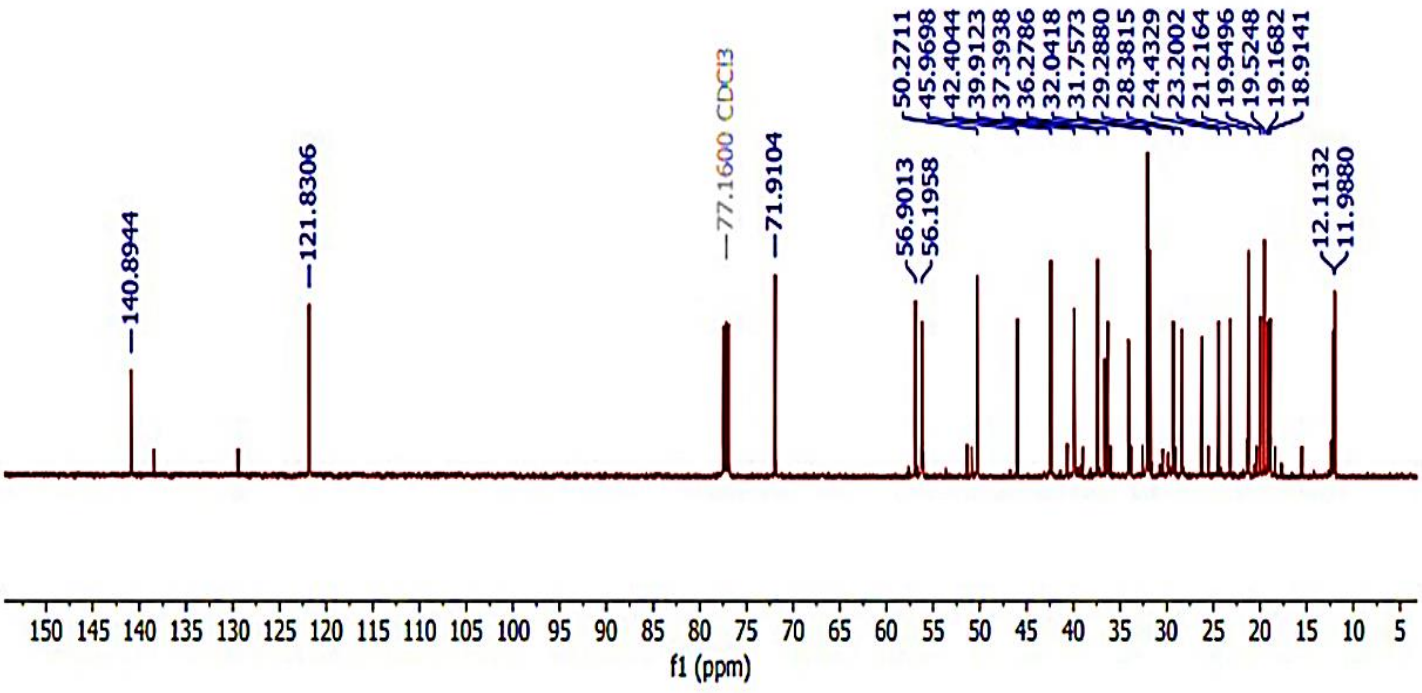
The proton H-6 (δ_H 5.33-5.34 ppm) is connected to the double bond of C-5 and C-6, and H-3 (δ_H 3.46-3.54 ppm) is a proton of methine that binds to C-O. The proton chemical shift at the H-3 position has a large value because of the influence of the electronegativity of the O atom pulling electrons from that position.

The ^{13}C-NMR spectrum shows that the isolated compound has 29 carbon atoms, and one peak is larger than the other peaks. This is characteristic of the peak, which has two C atoms with the same chemical shift, namely, at δ 32.04 ppm. The ^{13}C-NMR spectrum of the isolated compound was similar to the β-sitosterol spectrum with the molecular formula $C_{29}H_{50}O$, which was previously reported in a study [21]. The ^{13}C-NMR spectrum of isolated compound is shown in Figure 2.

Table 2: Comparison of ^{1}H-NMR and ^{13}C-NMR spectral data of isolated compound of the extract of *W. bifurcata* with *β*-sitosterol compound

Carbon number	Compound of *W. bifurcata*	Compound of *W. bifurcata*	*β*-sitosterol[21]	*β*-sitosterol[21]
	δ_H, ppm	δ_C, ppm	δ_H, ppm	δ_C, ppm
1	1.01-1.06, m	37.39	1.01-1.06, m 1.80-1.85, m	37.40
2	1.80-1.85, m	31.75	1.79-1.83, m 1.92-2.00, m	31.52
3	3.46-3.54, m	71.91	3.45-3.54, m	71.78
4	1.94-2.40, m	42.40	1.90-2.00, m	42.15
5	-	140.89	-	140.68
6	5.33-5.34, d	121.83	5.31-5.33, d	121.69
7	1.40-1.45, m 1.80-1.85, m	32.04	1.38-1.44, m 1.79-1.83, m	31.86
8	1.40-1.45, m	32.04	1.38-1.44, m	31.86
9	0.86-0.91, m	50.27	0.86-0.91, m	50.08
10	-	36.63	-	35.45
11	1.40-1.53, m	21.21	1.40-1.53, m	21.04
12	2.21-2.26, m	39.91	2.21-2.26, m	39.73
13	-	42.45	-	42.28
14	0.91-1.01, m	56.90	0.90-1.01, m	56.72
15	1.44-1.53, m	24.43	1.44-1.53, m	24.27
16	1.80-1.85, m	28.38	1.79-1.83, m	28.22
17	1.01-1.06, m	56.19	1.01-1.06, m	56.01
18	0.67, s	11.98	0.64, s	11.82
19	0.99, s	19.52	0.97, s	19.36
20	1.27-1.38, m	36.27	1.27-1.38, m	36.11
21	0.78, d	18.91	0.73; 0.75, d	18.74
22	0.95-1.01, m	34.07	0.95-1.01, m 1.20-1.26, m	33.90
23	1.01-1.10, m	26.21	1.01-1.10, m	26.00
24	0.83-0.88, m	45.96	0.83-0.88, m	45.78
25	1.61-1.69, m	29.28	1.61-1.66, m	29.06
26	0.83, d	19.94	0.82; 0.84, d	19.79
27	0.81, d	19.16	0.79; 0.81, d	19.00
28	1.20-1.30, m	23.20	1.20-1.28, m	23.01
29	0.90; 0.91, d	12.11	0.88; 0.90, d	11.94

Figure 2: The ^{13}C-NMR spectrum of isolated compound

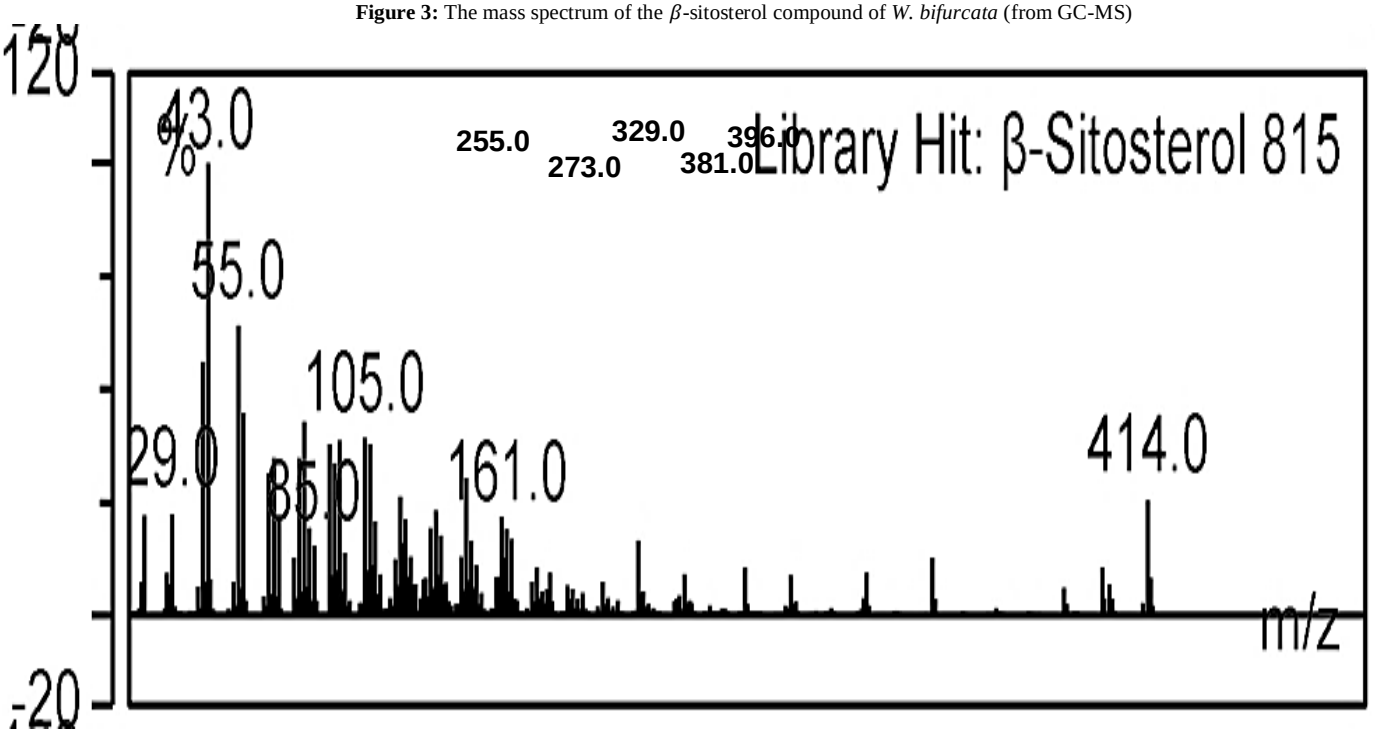


The chemical shift of the ^{13}C-NMR spectrum of the isolated compound revealed sp^3 carbon signal of methyl group sappeared at δ_C 11.98; 12.11; 18.91; 19.16; 19.52; and 19.94 ppm. The methylene and methine groups appeared at δ_C 21.21; 23.20; 24.43; 26.21; 28.38; 29.28; 31.75; 32.04; 34.07; 36.27; 37.39; 39.91; 42.40; 45.96; 50.27; 56.19; 56.90; 71.91; and 121.83 ppm. In addition, signals of 3 quaternary C atoms, namely; C-5, C-10, and C-13, appeared at δ_C 140.89; 36.63; and 42.45 ppm, respectively. The C-3 atom (δ_C 71.91 ppm) binds to the O atom in the OH group, whereas the C-5 (δ_C 140.89 ppm) and C-6 (δ_C 121.83 ppm) position on the C atom had a double bond.

Mass Spectroscopy Data

The results of the GC-MS characterization of the ethanol extract of *W. bifurcata* fruit had 14 compounds with oleic acid (18.39%) as a dominant compound and β-sitosterol (4.56%) which was suspected as the isolated compound. The mass spectrum of the β-sitosterol compound of the *W. bifurcata* fruit showed a peak at m/z 414, the mass spectrum of the compound is shown in Figure 3.

Figure 3: The mass spectrum of the β-sitosterol compound of *W. bifurcata* (from GC-MS)



The fragmentation pattern of the mass spectrum of the β-sitosterol compound showed that termination of the H_2O molecule at m/z 414 produced a compound with m/z 396. The compound with m/z 396 underwent CH_3 termination to produce the compound with m/z 381. The fragmentation of the compound with m/z 414 can also undergo cleavage (C_6H_{11} and H_2) to form a compound with m/z 329 and cleavage ($C_{10}H_{19}$ and H_2) to form a compound with m/z 273. Furthermore, the compound with m/z 273 formed a compound with m/z 255 via H_2O cleavage [22]. The fragmentation pattern of β-sitosterol is shown in Figure 4 [22].

Based on the ^{1}H-NMR, ^{13}C-NMR, and fragmentation pattern of the MS spectrum, the isolated compound obtained from the ethanol extract of *W. bifurcata* fruit was suspected as a β-sitosterol compound (pure isolate) with a molecular formula $C_{29}H_{50}O$ which has 1 double bond in its steroid framework and a melting point of 135-136℃. This is supported by the comparison of the spectrum of β-sitosterol that has been reported by previous studies.

The SPF Values of Extract, Subfraction, Pure Compound and Each Lotion Form of *W. bifurcata*

The ethanol extract of *W. bifurcata* at concentrations of 2%, 2.5%, 5%, 7.5% and 12% had SPF values of 36.921± 0.607; 37.018 ± 0.391; 38.434 ± 0.078; 38.660 ± 0.095; and 39.144 ± 0.450, respectively. The subfraction and subfraction lotion were also tested for sunscreen activity, subfraction C had the highest SPF value (37.079 ± 0.443). However, subfractions A (36.876 ± 0.052) and B (36.815 ± 0.067) had SPF values that were not significantly different from that of subfraction C. The SPF value of the extract was higher than that of the subfraction because the subfraction was a separation of compounds from the extract. The graphs of the SPF values of the extract and subfraction are shown in Figure 5.

The ethanol extract lotion of 2%, 2.5%, 5%, 7.5% and 12% was 28.107 ± 0.210; 29.215 ± 0.314; 35.686 ± 0.225; 36.050 ± 0.093; 37.039 ± 0.368, respectively. Furthermore, the SPF value of subfractions lotion A, B, and C was 26.506 ± 0.692; 25.798 ± 0.392; and 29.582 ± 1.100, respectively. The graphs of the SPF values of the extract lotion and subfraction lotion are shown in Figure 5.

Figure 4: The fragmentation of β-sitosterol

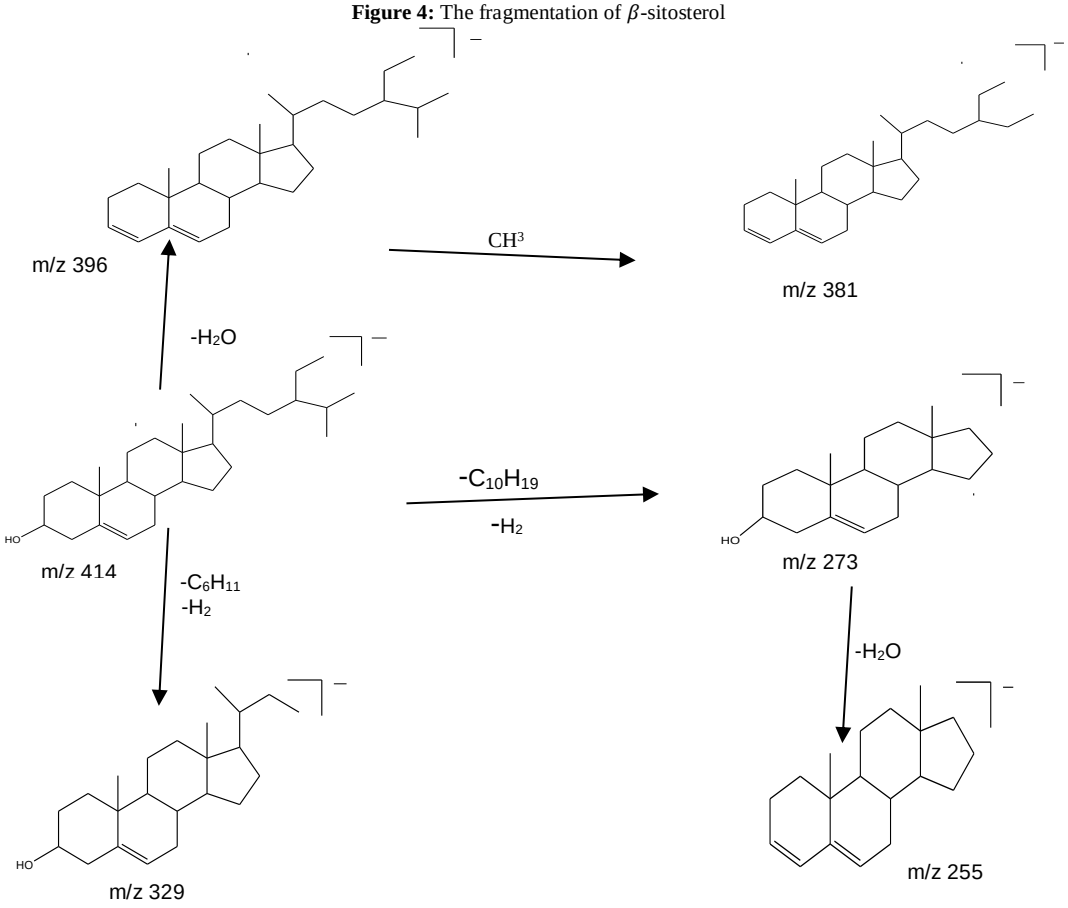
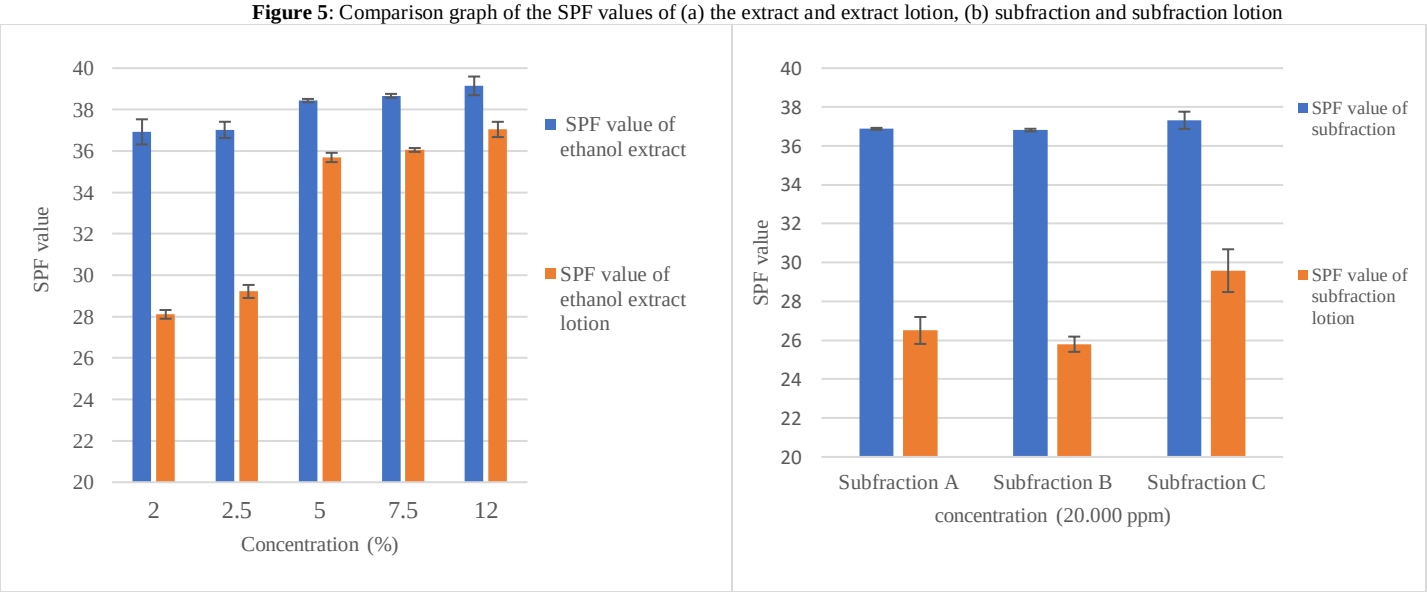


Figure 5: Comparison graph of the SPF values of (a) the extract and extract lotion, (b) subfraction and subfraction lotion



(a) (b)

The SPF value is influenced by the antioxidant activity of the extract. The IC_{50} value resulting from the antioxidant test of the ethanol extract of *W. bifurcata* fruit had sufficient antioxidant capacity so the SPF of each extract concentration and subfraction produced were in the ultra-category (SPF value > 15). The SPF of each extract concentration and subfraction of *W. bifurcata* fruit were higher than that of the lotion. This is because the ingredients in the lotion formulation contained in the extract lotion reduced sunscreen activity [6].

Further isolation of the subfraction A yielded a pure isolate. The pure isolate and the lotion were also tested for sunscreen activity. The pure isolate (9.104 ± 0.268) had a higher SPF value than the pure isolate lotion (6.091 ± 0.187). The SPF values of the pure isolate and pure isolate lotion are in the maximum and medium categories, respectively.

The Physical Properties of *W. bifurcata* Extract Lotion

Tests on the physical properties of the ethanol extract lotion at each concentration were performed to obtain the optimal sunscreen

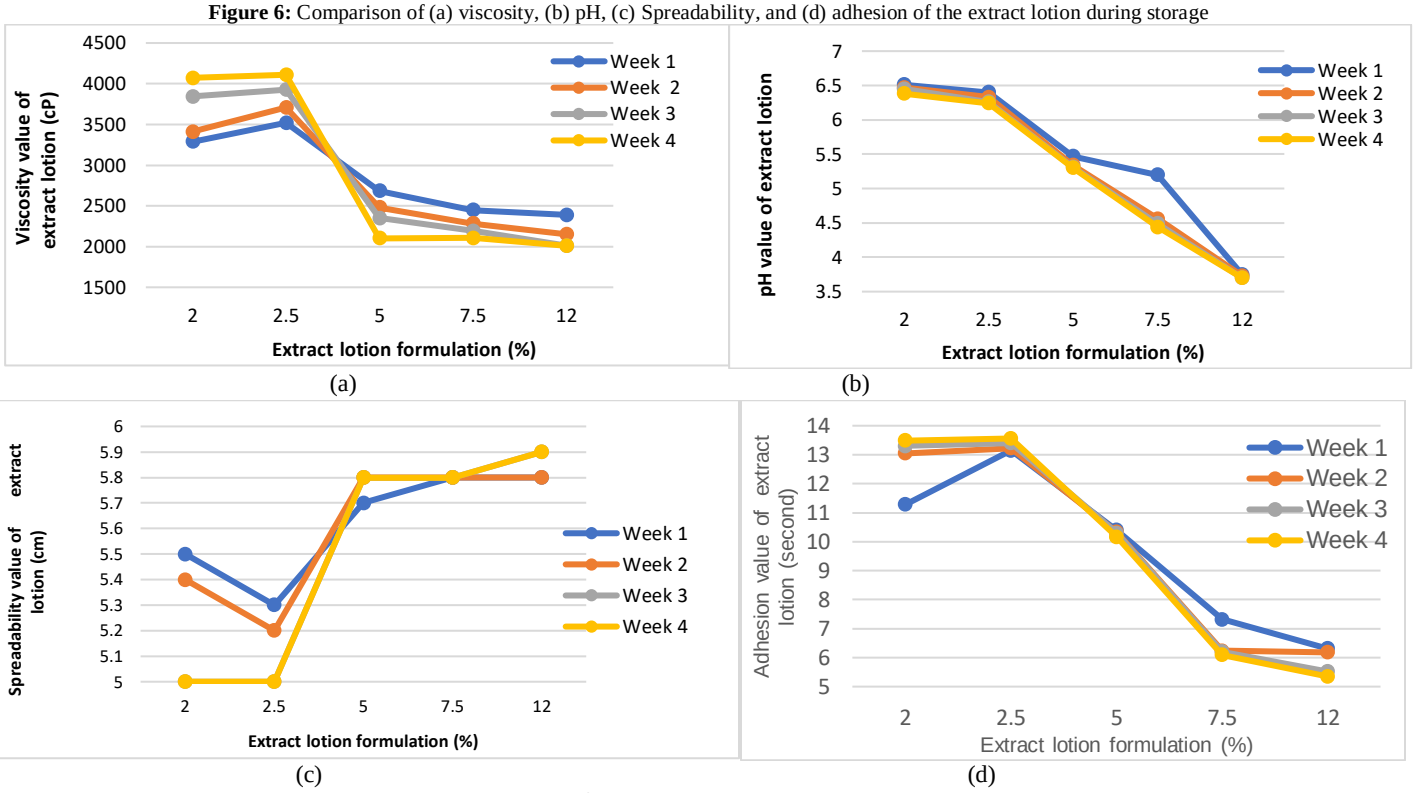
lotion formulation according to the Indonesian National Standard (SNI).

Emulsion-Type, Viscosity, pH, Spreadability, and Adhesion Test

The emulsion type test showed that the emulsion type of all concentrations of the ethanol extract lotion of *W. bifurcata* had oil in water type. The emulsion type test for 4 weeks remained on the oil in the water type, which indicated that the lotion was stable.

The results of the viscosity, pH, Spreadability, and adhesion tests showed that the extract lotion at each concentration did not change significantly for 4 weeks. Figure 6 shows the test results.

Figure 6: Comparison of (a) viscosity, (b) pH, (c) Spreadability, and (d) adhesion of the extract lotion during storage



The viscosity test showed that the concentrations of 5-12% decreased, which was influenced by the increasingly acidic pH. The viscosity values of the extract lotion are still in the range of 2009.3-4110.0 cP. All of these concentrations met the requirements for sunscreen as semi-solid preparations, namely in the range of 2000-5000 cP [23].

The pH values of the ethanol extract lotion at concentrations of 2-12% were in the range of 3.70-6.51. The ethanol extract lotion of *W. bifurcata* at concentrations of 2-7.5% was still in the pH range that complied with the quality requirements for sunscreen preparations, namely 4.5-8 [23].

The Spreadability at concentrations of 2-2.5% decreased due to the increased viscosity of the extract lotion. The test results showed the same Spreadability at weeks 3 and 4. The ethanol extract lotion of *W. bifurcata* at concentrations of 2-12% had Spreadability values in the range of 5-5.9 cm. It was in the range of good lotion Spreadability, which is 5-7 cm.

The adhesion test with a long time at a concentration of 2.5% and a short time at a concentration of 12%. The viscosity of each lotion also caused this. The adhesion of the ethanol extract lotion of *W. bifurcata* at concentrations of 2-12% was in the range of 05.35-13.56 s. The adhesion test of the lotion for 4 weeks at each concentration showed good adhesion. A good lotion has an adhesion of no less than 4 s [24].

Organoleptic Tests

The lotion was tested for color, fragrance, and texture by measuring the scale range 1: very like, 2: like, 3: little like, 4: dislike, and 5: very dislike. Tests on each formulation showed that the most preferred was 2% in terms of color, fragrance, and texture. The differences in panelist preference levels in the statistical tests for color, fragrance, and texture in each formulation's average value comparison are shown in Table 3.

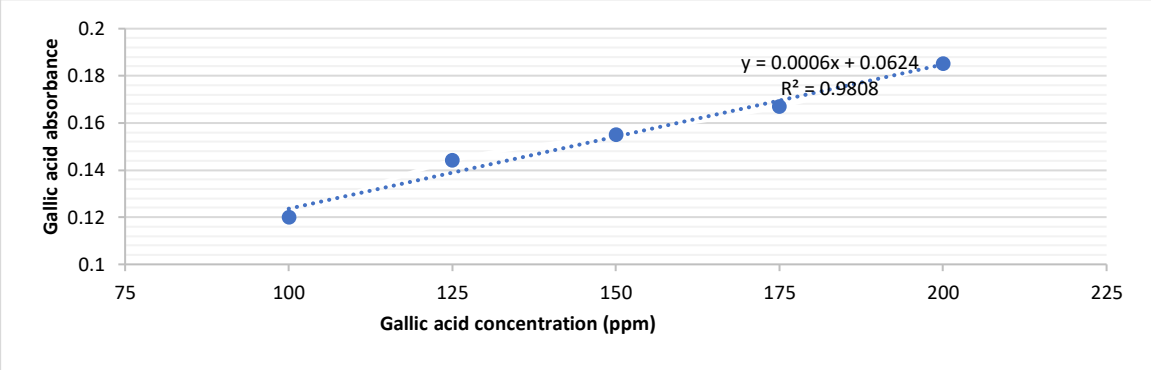
Table 3: Average values of the analysis of respondent's preference level for color, fragrance, and texture of extract lotion formulations

Sunscreen lotion formulation	Average preference level		
	Color	Fragrance	Texture
Formulation 2%	2.37	2.27	2.07
Formulation 2.5%	2.50	2.70	2.10
Formulation 5%	3.43	3.40	2.17
Formulation 7.5%	3.83	3.60	2.43
Formulation 12%	3.83	4.07	3.20

Total Phenolic Content

The total phenolic content of the ethanol extract of *W. bifurcata* fruit was determined from the standard gallic acid regression equation. Gallic acid standard calibration is presented in Figure 7.

Figure 7: Gallic acid standard calibration curve

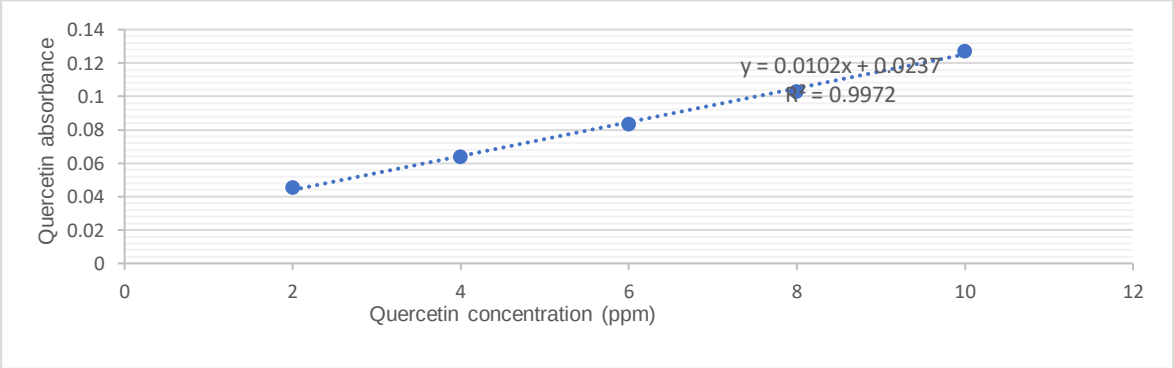


Based on the gallic acid standard calibration curve, the intercept value was 0.0624 and the slope value was 0.0006; thus, the standard curve equation was y = 0.0006x + 0.0624, and the coefficient of determination R^2 = 0.9808 with an average sample absorbance of 0.095. The total phenolic content of the ethanol extract *of W. bifurcata* fruit was 54.33 mg GAE/g. This follows the results of phytochemical tests, indicating phenolic compounds' presence.

Total Flavonoid Content

The total flavonoid content of the ethanol extract of *W. bifurcata* fruit was determined from the standard quercetin regression equation. Quercetin standard calibration is presented in Figure.

Figure 8: Quercetin standard calibration curve



Based on the quercetin standard calibration curve, the intercept value was 0.237 and the slope value was 0.0102; thus, the standard curve equation was y = 0.0102x + 0.0237 and the coefficient of determination R^2 = 0.9972 with an average sample absorbance of 0.06. The total flavonoid content of the ethanol extract of *W. bifurcata* fruit was 3.55 mg QE/g. This follows the results of phytochemical tests, indicating flavonoid compounds' presence.

Antioxidant Activity

Each variation in extract concentration was measured to determine the extract's ability to scavenge DPPH radicals, expressed in percent absorption. The classification of antioxidant activity is based on the IC_{50} value. The IC_{50} values produced from the ethanol extract of *W. bifurcata* fruit and ascorbic acid as positive controls were 217.95 ppm and 4.81 ppm, respectively. The ethanol extract was in the medium category in counteracting free radicals, namely in the range of 101-250 ppm.

CONCLUSION

The ethanol extract, subfractions, and the lotion of *W. bifurcata* fruit have shown the potential to protect the skin from UV exposure with an SPF value of > 15 (ultra), whereas the pure isolate, and the lotion revealed an SPF value of 9.104 ± 0.268 (maximum) and 6.09 ± 0.187 (medium), respectively. The physical properties of the extract lotion met the quality requirements for sunscreen preparations according to Indonesian National Standard (SNI 16-4399-1996), and the most preferred formulation of the organoleptic tests was 2%. The pure isolate obtained was suggested as β-sitosterol compound based on results of phytochemical tests and characterization data by GC-MS, ^{1}H-NMR, ^{13}C-NMR, and melting point experiments.

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Author contributions

MMH conducted the experiment and wrote manuscript; RN and MB wrote and revised the manuscript; YJ and M conducted the experiment and data analysis. The final version of the manuscript has been approved by all authors.

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