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Isolation of Caffeine from *Scurrula ferruginea* Jack Danser (Coffee Parasite) as a Sunscreen in Lotion Formula

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Abstract

BACKGROUND: The ultraviolet exposure is a major negative impacts on the human skin such as aging, sunburn, pigmentation, wrinkles, and skin cancer. One of the best ways to protect the skin from UV rays is using a sunscreen.

AIM: This research aims to determine the sunscreen activity of the extract, extract lotion, subfraction, and pure compound of Scurrula ferruginea and to determine the physical properties of the extract lotion.

METHODS: S. ferruginea was extracted by the maceration method using ethanol solvent. The sunscreen activity was evaluated using the spectrophotometric method and calculated by the Sun Protection Factor (SPF). The results were characterized using GC-MS and FTIR, the pure compound was suspected as a caffeine compound.

RESULTS: This caffeine has a good sunscreen activity with SPF values of 21.358 ± 0.011. The results of the SPF values of the extract, extract lotion, subfractions (A-E), and subfractions (A-E) lotion were range of 32.270 ± 0.227 to 38.171 ± 0.440; 21.358 ± 0.098 to 34.665 ± 0.315; 27.383 ± 0.407 to 34.719 ± 0.162; and 23.448 ± 0.147 to 32.039 ± 0.171 at concentrations of 2%, 2.5%, 5%, 7.5%, and 12%, respectively.

CONCLUSION: The SPF values of the extract, extract lotion, subfraction, and pure compound are included in the ultra-category. The results of physical properties of the extract lotion meet the standards set by Standard Nasional Indonesia (SNI) 1996.

Introduction

Sunlight is needed by humans as a source of light, energy, prevent, or treat bone disorders by activating provitamin D3 (7-dehydrocholesterol) found in the epidermis of the skin to become vitamin D3, but in excess conditions, sunlight can also have negative effects, especially on human skin [1]. The negative effects arise from exposure to UV radiation such as erythema, pigmentation and premature aging. Erythema (rash on the skin) occurs due to UV radiation at a wavelength of 290−320 nm, namely, UV-B radiation. Meanwhile, UV radiation at a wavelength of 320−400 nm is UV-A radiation which can cause pigmentation (darkness on the skin) [2]. Ultraviolet radiation can also cause very dangerous diseases such as melanoma skin cancer, which is the most aggressive and deadliest form of skin cancer [3].

Among the natural compounds that have the potential to act as sunscreens are compounds that have conjugated double bonds [1], and have antioxidant activity [4]. There are many plants that have chemical compounds with conjugated double bonds, such as *Artocarpus camansi* [5], [6], [7], *Ficus racemosa* [8], and *Morus alba* [9]. Our recent study of the *Artocarpus heterophyllus* showed sunscreen activity with ultra-category [10]. One source of natural antioxidants is found in the *S. ferruginea* (coffee parasite) plant from the Loranthaceae family. The results of the previous studies reported that *S. ferruginea* contains secondary metabolites of alkaloids, flavonoids, saponins, phenolic, and steroids [11]. Ethnobotany of *S. ferruginea* has been used as an anti-cancer drug [12], anti-inflammatory, diabetes [13], antimicrobial [14], and antioxidant [12], [15]. The previous studies have shown that the methanol extract of *S. ferruginea* stem has antioxidant activity with IC_{50} values of 27.81 ppm [12], and acetone extract from *S. ferruginea* leaves has antioxidant activity with IC_{50} values of 7.41 ppm [15]. The results of these studies proved that the *S. ferruginea* plant had very strong antioxidant activity. The presence of antioxidant compounds in sunscreens will increase photoprotective properties, thereby preventing the emergence of various diseases, including skin diseases caused by UV radiation [16]. The stronger the antioxidant activity, the greater the sunscreen's ability to protect the skin.

Subjects and Methods

Plant materials

The samples used in this study were all parts of the *S. ferruginea* plant from Bener Meriah Regency, Aceh Province. The plant was collected in January 2020. Plant authentication was conducted in Herbarium Department of Biology, Universitas Syiah Kuala.

Lotion-making materials

Cetyl alcohol (Merck), stearic acid (Merck), lanolin (Merck), glycerin (Merck), methyl parabens (Merck), triethanol amines (Merck), aqua distillates, *n*-hexane (Merck), ethanol 96% (Merck) and methanol (Merck), purchased from the Rudang store in Medan, North Sumatra.

Generals

Characteristics of the extract were measured using GC-MS QP 2010 Ultra (Shimadzu, Japan), FTIR spectrophotometer (Shimadzu, Japan), UV-1240 UV-Vis Spectrophotometer (Shimadzu, Japan), Thin Layer Chromatography (TLC) using silica gel G60-F_{254} (Merck), electrical balance (Mettler Toledo, Japan), rotary evaporator (Butchi R-300), pH meter (710 A Thermo electron Orion), viscometer (Thermo scientific Haake viscotester c), and scatter power test equipment, and glassware.

Phytochemical screening

The method used for testing the phytochemicals can be found in phytochemical methods, a guide to modern techniques of plant analysis [17].

Alkaloids identification

The extract was added with 0.05 N ammonia-chloroform solutions. The liquid phase was collected into a test tube. Added 5 mL of HCL 0.5 N and shaken. The mixture would be in two layers. The top layer is a solution in HCL and the bottom layer is a solution in chloroform. The solution in HCL was tested with three reagents. The test with Mayer's reagent was indicated by the formation of a yellow precipitate. Wagner's reagent was indicated by the formation of a brown precipitate, and Dragendorff's reagent is indicated by the formation of an orange or red precipitate [17].

Steroids and terpenoids identification

The chloroform solution (from Alkaloids identification) was added with a few drops of Liebermann-Burchard. The color changes into red indicated terpenoids while a green or blue color indicated steroids [17].

Flavonoids identification

As much as 0.5 g of sample was extracted in 5 mL of methanol and then heated through the test tube. The extract was added some drops of HCL (concentrated grade) and magnesium powder. The color changes into pink or purple indicated flavonoids [17].

Saponins identification

The extract was added with Aquadest and shaken in a test tube for 5 min. A foam forming for about 5 min indicated saponins [17].

Scurrula ferruginea Jack Danser plant extraction

As much as 2.3 kg of dry *S. ferruginea* plants were macerated using *n*-hexane solvent for 3 × 24 h. Maceration was repeated until a clear filtrate was obtained. The filtrate was filtered; the residue was dried and then macerated again using ethanol for 3 × 24 h and filtered. The filtrate of ethanol was evaporated using a vacuum rotary evaporator. The ethanol extract was tested for phytochemical analysis, sunscreen activity by determining the Sun Protection Factor (SPF) values and characterized using GC-MS.

Isolation procedure to get a pure compound

As much as 18 g of ethanol extract was fractionated using a gravity chromatography column. The separation of compound components was carried out by the elution gradient method. The eluent system used was *n*-hexane and ethyl acetate with a ratio of 100:0; 95:5; 90:10; 85:15; 80:20; and 70:30, and obtained fractions. All fractions were monitored by TLC. The fractions that had the same mode pattern were combined to obtain subfractions A, B, C, D, and E. Subfraction C was rechromatographed again using a gravity chromatography column with an eluent of *n*-hexane 100%. Then their purity was tested three different eluents, *n*-hexane: chloroform (8:2), *n*-hexane: ethyl acetate (7:3), and *n*-hexane: acetone (6:4). The yield of TLC chromatogram showed one stain pattern that indicated as a pure compound.

Making sunscreen lotion

The preparation of lotion formulations with active ingredients of extracts and ethanol subfraction of *S. ferruginea* plants was carried out based on the provisions of the Indonesian Pharmacopoeia which

had been carried out in a previous study [18] and then modified. The amount of the ethanol extract added to the lotion base formulation is listed in Table 1.

Table 1: The formula of lotion preparation (with modification) [18], [19]

Serial number	Material	Composition of lotion materials (%)				
I	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	Extract/subfraction/pure compound	2	2.5	5	7.5	12
	Glycerin	2	2	2	2	2
III	Methylparaben	0.1	0.1	0.1	0.1	0.1
	TEA	0.75	0.75	0.75	0.75	0.75
	Aquades	90.65	90.15	87.65	85.5	80.65

TEA: Triethanolamine.

We weighed all the necessary ingredients, then part I (Table 1), materials were inserted into a porcelain cup and melted over a water bath to a temperature of 70°C. Part III (from Tabel 1) was dissolved in hot Aqua. Then part III was inserted in porcelain in a hot state, then added part I into section III with constant stirring until the temperature drops. At 45°C, added ethanol extract with the concentrations: 2%, 2.5%, 5%, 7.5%, and 12% [19], [20], that has been mixed with glycerin (II, from Table 1), while stirring until homogeneous. It is then fed into the appropriate container [21].

Measurement of SPF (Sun Protection Factor) values

The method for the absorption of sunscreen was determined based on spectrophotometric analysis [22]. A lotion sample weighing 0.5 g was dissolved in 25 mL of 96% ethanol (20,000 ppm). The absorbance of samples was measured with a UV spectrophotometer every 5 nm over a wavelength range of 290 nm–320 nm with 96% ethanol as a blank. Calculation of SPF values according to using the following equation [23].

$$SPF = CF\sum_{290}^{320} EE(\lambda) \times I(\lambda) \times A(\lambda)$$

Where: EE is erythemal effect spectrum; I is solar intensity spectrum; Abs is absorbance of sunscreen product; CF is correction factor (= 10), the values of EE × I are constants [22]. The relationship between erythemogenic effect and radiation intensity at each wavelength is listed in Table 2 [22].

Table 2: Relationship between erythemogenic ffect and radiation intensity at each wavelength

Wavelength (nm)	EE×I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

The examination of the lotion

The examination of the lotion was carried out on the sunscreen activity as well as the physical properties of the lotion, that is, pH [24], the power of spreadability [25], type of emulsion [26], viscosity [27], and the power of adhesiveness [28].

Determination of pH

5 g of the lotion was added with 45 ml of water, and then dissolved. The pH value of the lotion is measured using a pH meter [24].

Determination of spreadability

1 g of the lotion was placed between round glass with a diameter of 20 cm. then put a load of 100 g on the glass, let stand for 1 min then measure the constant diameter [25].

Type of emultion test

3 g of the lotion was tested with ammeter. If the ammeter produces current then the lotion emulsion type is o/w (oil in air) [26].

Determination of viscosity

The viscosity was evaluated using viscometer with LV-64 spindle. The lotion was directly immersed into the spindle and the viscosity was measured [27].

Determination of adhesiveness

5 g of the lotion placed between glass objects. Then pressed with a load of 500 g for 5 min. The object glass then mounted on the test equipment that is given a load weighing 80 g then recorded the time required for the two glass objects to separate [28].

Results and Discussion

Scurrula ferruginea Jack Danser plant extraction

A total of 2.3 kg of crushed dried samples of *S. ferruginea* plants were extracted using the maceration method, and obtained 16.972 g (0.727%) of *n*-hexane extract and 48.419 g (2.105%) of the ethanol extract.

Phytochemical test results

Phytochemical test results of the ethanol extract of *S. ferruginea* are listed in Table 3.

The presence of alkaloid secondary metabolites is characterized by the formation of brown deposits with Mayer's reagent, white deposits with

Table 3: Phytochemical test results of ethanol extract of *Scurrula ferruginea*

Secondary metabolites	Test method	Results
Alkaloids	Mayer	+
	Wagner	+
	Dragendorff	+
Steroids	Liebermann-Burchard	–
Terpenoids	Liebermann-Burchard	–
Saponins	Water and shaking	+
Flavonoids	0.5 g Mg dan HCl	–
Phenolic	$FeCl_3$	+

Wagner's reagent and red deposits with Dragendorff's reagent. Furthermore, the ethanol extract of the *S. ferruginea* plant was tested for its sunscreen activity and formulated in a lotion preparation.

The SPF Values of the extract and extract lotion of S. ferruginea

The results of the SPF values of the ethanol extract and extract lotion obtained are listed in Table 4.

Table 4: Sun protection factor values of ethanol extract and extract lotion of *Scurrula ferruginea*

Concentration	SPF values of extract	SPF values of extract lotion
2.0%	32.270 ± 0.227	21.358 ± 0.098
2.5%	33.414 ± 0.280	23.042 ± 0.029
5.0%	35.360 ± 0.502	32.357 ± 0.326
7.5%	37.576 ± 0.466	33.333 ± 0.398
12.0%	38.171 ± 0.440	34.665 ± 0.315
Negative control	-	0.728 ± 0.048
Positive control	-	33.229 ± 0.316

SPF: Sun protection factor.

It can be seen that the ethanol extract of the *S. ferruginea* plant at a concentration of 12% has the highest SPF values of 38.171 ± 0.440. Meanwhile, at a concentration of 2%, the SPF values are the lowest, which is 32.270 ± 0.227. This is because the content of secondary metabolites at a concentration of 12% is more, so that these compounds produce high absorbance values. The greater the absorbance obtained, the higher the SPF values. The SPF values obtained from each concentration is in the ultra-category (SPF >15) [29]. The comparison graph of the SPF values of the extract and extract lotion is presented in Figure 1.

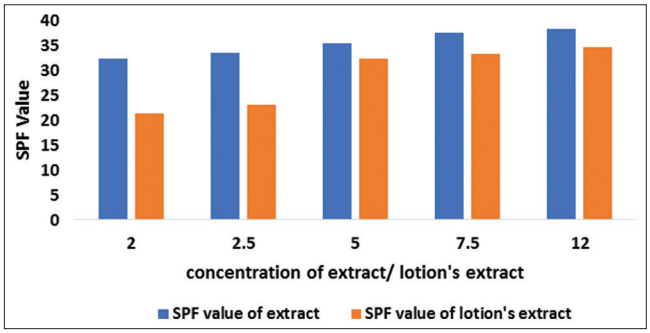


Figure 1: The comparison graph of the SPF values of S. ferruginea extract and extract lotion

The SPF values in the extract lotion are smaller than the extract SPF values. This is due to the addition of chemicals in the lotion formulation which causes the mobility of the compounds in the extract to be limited, so that sunscreen activity decreases [10].

Physical properties of S. ferruginea plant extract lotion

Emulsion type

The emulsion type test was carried out using an amperemeter. The type of emulsion lotion of *S. ferruginea* plant extract was oil in water (o/w). During the 1st to 4th-week, storage showed the stability of the lotion. However, at a concentration of 12% from week 3 to week 4, the emulsion type changed to w/o (water in oil). This is due to the oxidation of the components in the lotion, namely, the oil phase contained in the lotion. Hence, it can be concluded that the ethanol extract lotion at a concentration of 12% is unstable [28]. A good emulsion type test is oil in water (o/w) because it is easier to clean with water [30].

pH test

The pH test of the lotion was measured with a pH meter. Extract lotion at a concentration of 2% had the highest pH values which was 7.801 and at a concentration of 12% had the lowest pH values, which was 6.082. The pH observations indicated that the ethanol extract of the *S. ferruginea* plant was acidic. So that the greater the concentration of the concentrated ethanol extract of the *S. ferruginea* plant in the lotion, the lower the pH values obtained. Graph of pH values against lotion storage time is presented in Figure 2.

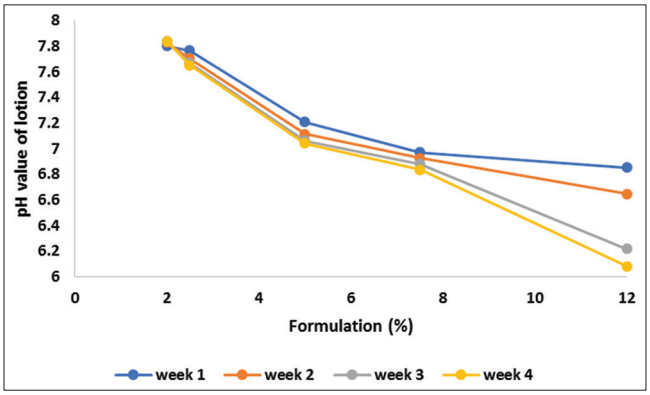


Figure 2: Comparison of pH values to lotion storage time

The pH values measurements at a concentration of 2%–7.5% during week 1 to week 4 experienced a significant reduction; whereas at a concentration of 12% the pH of the lotion had insignificant decrease. However, the pH values of the ethanol extract of *S. ferruginea* plant lotion had met the requirement, which is in the range 4.5–8 (SNI 16-4399-1996 [31].

Lotion viscosity

The extract lotion at a concentration of 12% had the highest viscosity values, which was 4850 cP and at

a concentration of 2% had the lowest viscosity values, which was 1977 cP. However, the extract lotion at a concentration of 12% decreased from week 2 to week 4. The decrease in viscosity values was probably due to the glycerin in the lotion formulation which decomposes due to heating [32]. The decrease in viscosity can also occur due to air and temperature factors [28]. A graph of the comparison of the viscosity values to the storage time in each lotion formulation is presented in Figure 3.

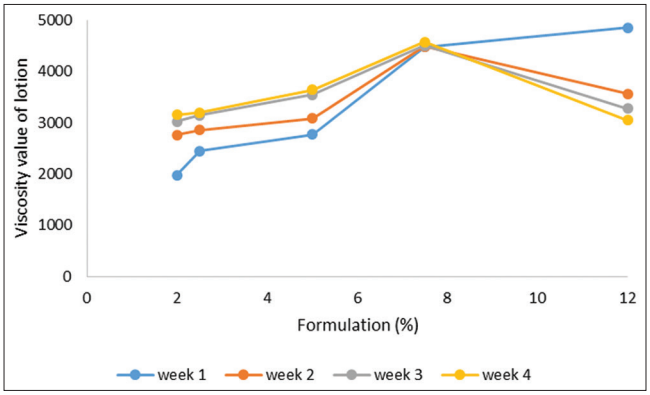


Figure 3: Comparison of viscosity value to storage time for each lotion formulation

The viscosity value of the ethanol extract lotion of *S. ferruginea* plant had an insignificant increase. The viscosity values at a concentration of 12% during week 2 to week 4 resulted in inconsistent data, but in general the viscosity values of the ethanol extract of *S. ferruginea* plant had met the requirement, namely, in the range of 2,000–50,000 cP (SNI 16-4399 -1996) [31].

Spreadability test

The spreadability of a lotion can be said to be good if the lotion can be easily applied to the skin surface, so that the active substances contained in the ethanol extract of the *S. ferruginea* plant can be distributed properly [28]. The extract lotion at a concentration of 12% had the lowest values, which was 4.40 cm and a concentration of 2% obtained the highest values, which was 6.25 cm. In general, the spreadability test of the extract lotion from week 1 to week 4 decreased. This was because the viscosity of the lotion had increased. The higher the viscosity values, the lower the spreadability of the lotion. The graph of the comparison of the values of the spreadability to the storage time of the lotion is presented in Figure 4.

The spreadability of the ethanol extract lotion was in the range of 4.4–6.25 cm. A good lotion has a spreadability in the range of 5.4–6.4 cm (SNI 16-4399-1996) [31]. Based on the data above, the spreadability of the lotion that met the requirement was at a concentration of 2% to 7.5% which had a spreadability in the range of 5.00–6.25 cm, while a lotion at a concentration of 12% did not met the requirement.

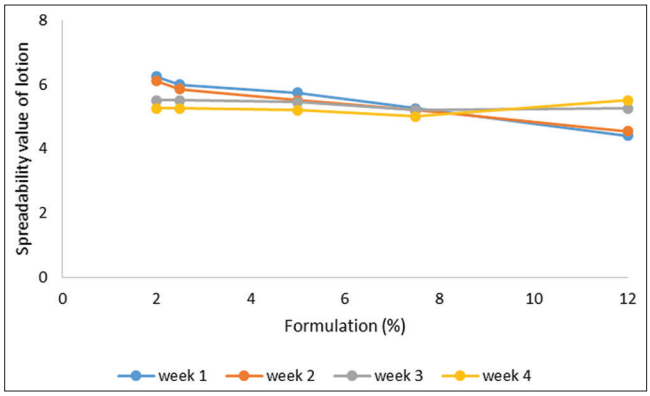


Figure 4: Comparison of the spreadability values to the storage time of the lotion

Adhesion power of the lotion

The extract lotion at a concentration of 12% obtained the highest adhesion values, which was 20.95 s and a concentration of 2% had the lowest adhesion values, which was equal to 12.03 s. In general, the adhesion test of the extract lotion from week 1 to week 4 had increased, but extract lotions at a concentration of 12% decreased from week 2 to week 4. Adhesion is directly proportional to viscosity. The lower the viscosity values of the lotion, the lower the adhesion obtained. The graph of the values of adhesion to the storage time of the lotion is presented in Figure 5.

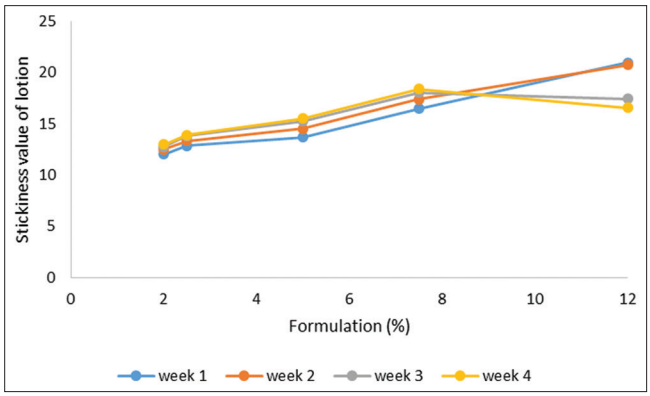


Figure 5: Comparison of adhesion values to lotion storage time

The adhesion values at a concentration of 12% showed inconsistent data. A good lotion has more than 4 s of adhesion [28]. Based on the data above, the adhesion of the lotion at a concentration of 2%–12% fulfilled the requirement, which was in the range of 12.3–20.95 s.

Fractionation of ethanol extract of S. ferruginea

18 g of ethanol extract was fractionated using a gravity chromatography column. The separation of compound components was carried out by the elution gradient method. The eluent system used was *n*-hexane and ethyl acetate with a ratio of 100:0; 95:5; 90:10; 85:15; 80:20, and 70:30, obtained as many as 114 fractions. All fractions were monitored by TLC.

The fractions that had the same mode pattern were combined to obtain five subfractions A, B, C, D, and E. The TLC chromatogram of subfractions A, B, C, D, and E is presented in Figure 6.

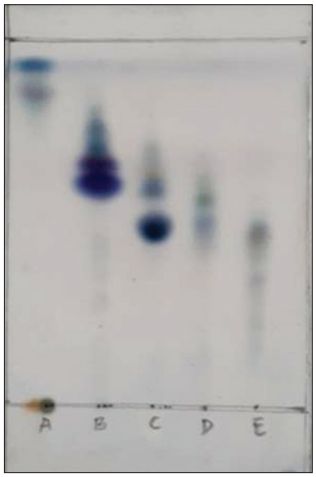


Figure 6: The TLC chromatogram of subfractions A, B, C, D, and E

The subfraction C (Figure 6) which was relatively pure was rechromatographed to obtain a pure compound. Then, all the subfractions were tested for sunscreen activity and formulated in a lotion preparation.

The SPF Values of the subfractions and subfractions lotion of S. ferruginea

The SPF values of the subfractions and subfractions lotion of *S. ferruginea* plants were determined using the same test procedure as the determination of the SPF values of the extract lotion, that is, each subfraction and subfractions lotion was prepared in a concentration of 20,000 ppm. The results of the SPF values of subfractions and subfractions lotion of *S. ferruginea* plants are listed in Table 5.

Table 5: Sun protection factor values of subfractions and subfractions lotion

Subfraction	SPF values of subfraction	SPF value of subfraction lotion
A	30.677 ± 0.131	27.600 ± 0.039
B	34.719 ± 0.162	32.039 ± 0.171
C	33.911 ± 0.262	30.183 ± 0.334
D	30.109 ± 0.398	26.457 ± 0.210
E	27.383 ± 0.407	23.448 ± 0.147
Negative control	-	0.728 ± 0.048
Positive control	-	32.153 ± 1.121

SPF: Sun protection factor.

The subfraction B had the highest SPF values, which was 34.719 ± 0.162 and subfraction E had the lowest SPF values, which was 27.383 ± 0.407. A high SPF values indicates the existence of an active compound that can protect the skin from UV rays. Subfraction B lotion had the highest SPF values, which was 32.039 ± 0.171 and subfraction E lotion had the lowest SPF values, which is 23.448 ± 0.147. Based on the data obtained, the subfraction lotion in each subfraction is included in the ultra-category [29]. The results that have been obtained proved that the subfraction lotion of *S. ferruginea* contains compounds capable of protecting the skin from UV rays, namely compounds that have chromophore groups or conjugated double bonds. The comparison graph of the SPF values of the subfraction and subfraction lotion of the ethanol extract of the *S. ferruginea* plant is presented in Figure 7.

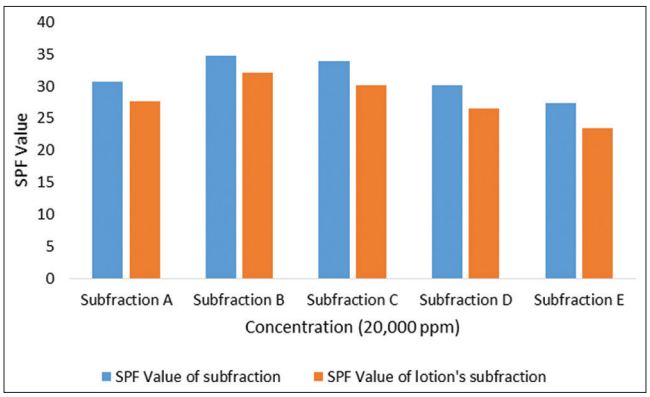


Figure 7: The comparison graph of the SPF values of subfraction and subfraction lotion of S. ferruginea plant ethanol extract

The SPF values of the subfraction lotion were lower than the SPF values of the subfractions, this is due to the addition of chemicals that can reduce lotion activity [10].

Isolation of subfraction C

About 0.1 g of subfraction C was rechromatographed again using a gravity chromatography column with an eluent of *n*-hexane 100%. The fractionation results were obtained as much as 9 fractions. The TLC chromatogram of the fractions is presented in Figure 8.

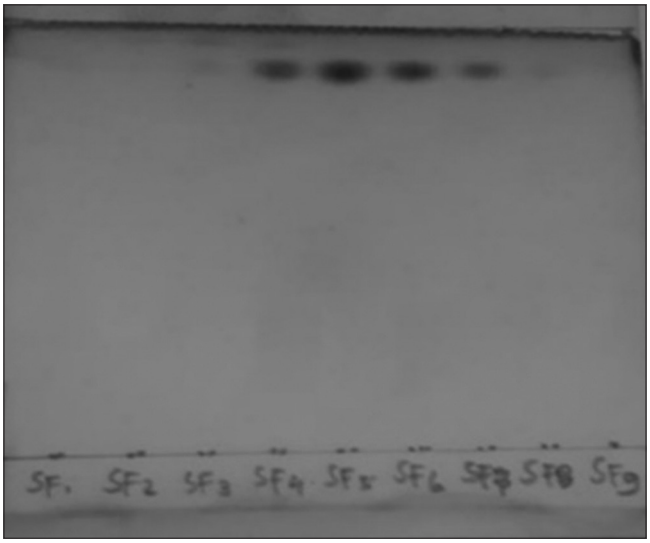


Figure 8: The TLC chromatogram of fractions from subfraction C

Fractions 5–7 which are relatively pure were combined into sub-subfractions 5–7 (SSF5-7), then their purity was tested with three different eluents, n-hexane: chloroform (8:2), n-hexane: ethyl acetate (7:3), and n-hexane: acetone (6:4). The yield of TLC chromatogram showed one stain pattern that indicated

as a pure compound. The Rf values obtained for the n-hexane: ethyl acetate (7:3) and n-hexane: acetone (6:4) eluent system were 0.60 and 0.76, respectively.

The pure compound SSF5-7 then formulated in a lotion preparation, tested for its sunscreen activity and phytochemical analysis. The activity of sunscreen lotion of pure compound SSF5-7 was carried out by comparing it with positive control (commercial sunscreen lotion). The SPF values of sunscreen lotion of pure compound SSF5-7 are listed in Table 6.

Table 6: Sun protection factor values of sunscreen lotion of pure compound sub-subfractions 5-7

Sample	SPF values
Positive control	30.931 ± 0.023
Pure compound SSF5-7	21.358 ± 0.011

SPF: Sun protection factor, SSF5-7: Sub-subfractions 5-7.

The SPF values of the pure compound SSF5-7 are lower than the SPF values of positive control, which was 21.358 ± 0.011. This is because there are only a few chromophore groups in the pure compound SSF5-7, so it did not produce high sunscreen activity like the positive control. However, the SPF values of pure compound SSF5-7 were included in the ultra-category (SPF >15) [29].

The results of phytochemical analysis on pure compound SSF5-7, positive for the presence of alkaloids which was indicated by the formation of red deposits after being reacted with Dragendorff's reagent. The type of alkaloid was suspected as a caffeine compound which is strengthened by GC-MS and FTIR.

Characterization of pure compound SSF5-7 with mass spectrometry (MS)

The spectrum of mass spectrometry of the pure compound SSF5-7 is presented in Figure 9.

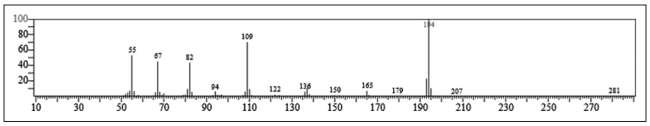


Figure 9: The spectrum of mass spectrometry of the pure compound SSF5-7

The spectrum of mass spectrometry of the pure compound SSF5-7 showed the peaks of m/z: 194, 179, 165, 150, 136, 122, 109, 94, 82, 67, and 55. The peaks of the pure compound SSF5-7 showed similarity to the peak of the caffeine compound. The characteristic of the caffeine compound is found in the fragment of m/z 194 as a base peak (100%) [33]. The fragmentation pathways of the caffeine compound are presented in Figure 10.

Characterization of pure compound SSF5-7 with fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum supported the pure compound SSF5-7, which showed the absorption of (N-H) amide stretching vibration at a wavenumber of

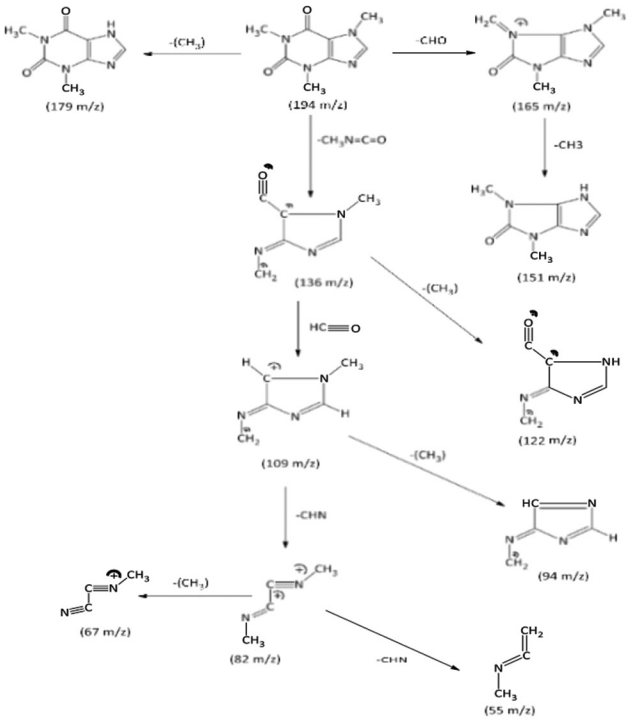


Figure 10: The fragmentation pathways of the caffeine compound [33]

3421 cm^{-1}. The aromatic (C-H) stretching vibration at a wavenumber of 3088 cm^{-1}. The aliphatic (C-H) vibration at the wavenumbers of 2936 and 2860 cm^{-1}. At the wavenumbers of 1645 and 1707 cm^{-1} showed two carbonyl groups (C=O) amide. The absorption at 1452 cm^{-1} showed aromatic carbon-carbon double-bound (C=C), and the absorption at 1375 cm^{-1} indicated stretch vibration (C-N) amine. The infrared (IR) spectrum of the pure compound SSF5-7 is presented in Figure 11.

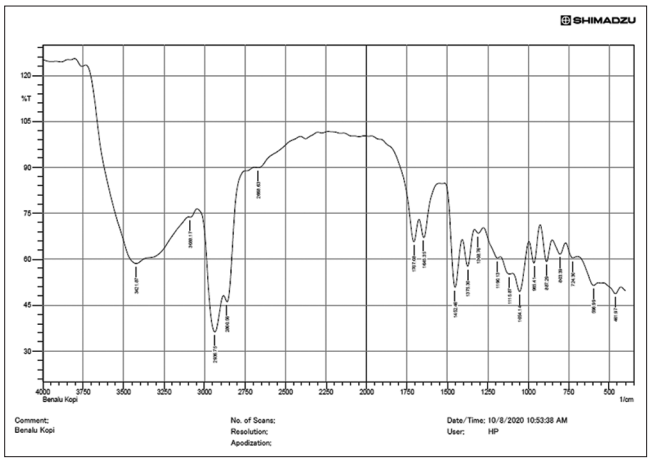
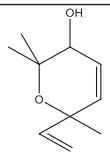
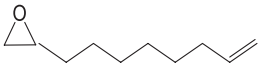
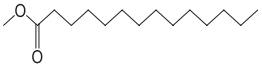
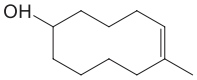
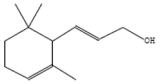
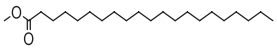
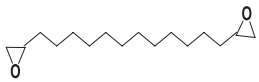
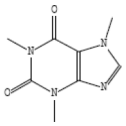
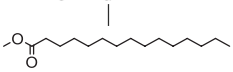
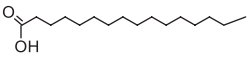
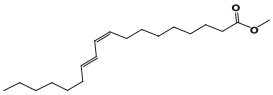
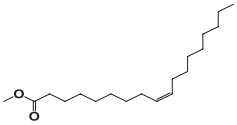
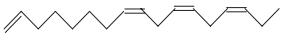
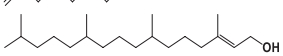
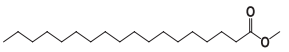


Figure 11: The infrared (IR) spectrum of the pure compound SSF5-7

The pure compound SSF5-7 as a similarity to the caffeine compound, so that the structure of the pure compound SSF5-7 was suspected as a caffeine compound. The structure of the caffeine compound is presented in Figure 12.

Based on the literature, it is known that caffeine or 1,3,7-trimethylxantin is a xanthine alkaloid compound in the form of crystals and a bitter taste

Table 7: Chemical compounds in ethanol extract of *Scurrula ferruginea* (from gas chromatography-mass spectrometry)

Serial number	Area	Similarity (%)	Name	Structure
1	0.75	78	(3R,6R)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	
2	0.92	79	Oxirane, (7-octenyl)-	
3	0.79	89	Hexadecanoic acid, 15-methyl-, methyl ester	
4	1.66	75	6-Methyl-cyclodec-5-enol	
5	1.20	78	2-Propen-1-ol, 3-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	
6	0.56	85	Heneicosanoic acid, methyl ester	
7	0.80	78	1,2-15,16-Diepoxyhexadecane	
8	18.71	96	Caffeine	
9	28.08	96	Hexadecanoic acid, methyl ester	
10	6.10	93	n-Hexadecanoic acid,	
11	5.99	91	Methyl 9-cis, 11-trans-octadecadienoate	
12	6.90	87	9-Octadecenoic acid (Z)-, methyl ester	
13	7.62	86	1,8,11,14-Heptadecatetraene, (Z, Z, Z)-	
14	15.11	94	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	
15	4.81	94	Methyl stearate	

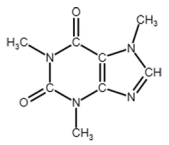


Figure 12: Structure of caffeine compound

that works as a psychoactive stimulant and mild diuretic [34]. Caffeine is a white alkaloid with the chemical compound formula C8H10N4O2, and the building formula 1,3,7-trimethylxanthine. Caffeine has a similar chemical structure to three alkaloid compounds, namely, xanthine, theophylline, and theobromine [35]. Caffeine melts at 227−228°C (anhydrous) 234−235°C (monohydrate). Caffeine can be found in several plant species, which is a natural pesticide, caffeine is found in newly grown plants [36]. Robusta coffee seed extract has antioxidant activity with IC50 of 54.14 ppm [37]. Robusta coffee (Coffea cenephora ex froehner) leave from ethyl acetate extract had a potential as a sunscreen with an SPF values of 19.82 [38]. The existence of caffeine is supported by GC-MS.

The results of the characterization of the ethanol extract of *S. ferruginea* parasite by gas chromatography (GC) are presented in Figure 13.

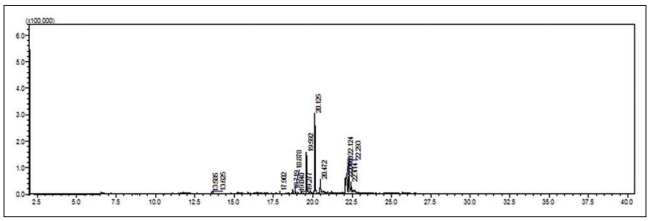


Figure 13: Chromatogram of the ethanol extract of the S. ferruginea plant by gas chromatography (GC)

Chemical compounds from GC were being analyzed with the NISTI Lab. 4 Library in mass spectrometry (MS). The chemical compounds contained in the ethanol extract of *S. ferruginea* with the composition are listed in Table 7.

The ethanol extract of *S. ferruginea* contains 15 chemical compounds. Among the 15 compounds, there were two abundant compounds, namely, caffeine (area: 18.71%; retention time: 19.592 min; similarity 96%) and hexadecanoic acid (area: 28.08%; retention time: 20.125 min; similarity 96%).

Conclusion

The ethanol extract of *S. ferruginea* plant can be formulated as a sunscreen lotion with an SPF values in the ultra-category (SPF > 15). The extract, subfraction, pure compound, and their lotions have the potential to protect the human skin from UV rays. The results of the phytochemical test, GC-MS, and FTIR showed that the pure compound SSF5-7 was suspected as a caffeine compound. This caffeine can be applied as a sunscreen with SPF values of 21.358 ± 0.011 (ultra-category).

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