Indonesian Journal of
Chemical Analysis

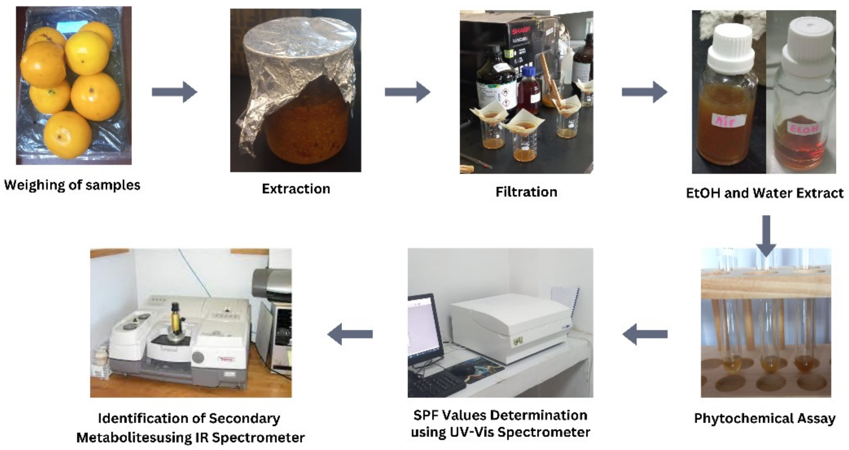
Homepage: https://journal.uii.ac.id/IJCA

In Vitro Activity of Ethanol Extract of Sour Eggplant Fruit (*Solanum ferox*) as an Active Ingredient in Sunscreen Formulations

Septaria Yolan Kalalinggi, Rasidah*, Yuliana

Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Palangka Raya, Palangka Raya, 73111, Indonesia
*corresponding author: rasidah@mipa.upr.ac.id
DOI: 10.20885/ijca.vol7.iss1.art8

GRAPHICAL ABSTRACT



ARTICLE INFO

Received : 13 December 2023
Revised : 15 February 2024
Published : 31 March 2024
Keywords : sour eggplant, phytochemical test, Sun Protector Factor (SPF), UV light

ABSTRACT

Solanum ferox is a typical Central Kalimantan plant widely used by Dayak's community as a vegetable. The study aims to determine the ethanol extract of sour eggplant activities by measuring its SPF in vitro and identifying secondary metabolite contents. The sour eggplant is extracted by maceration using 96% ethanol solvent and then bound with an evaporator. The secondary metabolite groups of ethanol extract were identified using phytochemical screening. The identification results indicate the presence of flavonoid, terpenoid, steroid, and phenolic compounds. Then, the ethanol extract was fractionated with a water solvent, and its functional cluster was identified using the IR spectrum. IR spectral identification results indicate the presence of OH, C-O, C=C olefin, C -O ether, and aliphatic CH functional groups. (CH_2 and CH_3) SPF values were determined by measuring the absorption of ethanol and water extracts in vitro using UV-Vis spectrophotometry at UV-B wavelengths of 290-320 nm. In vitro testing has been conducted as a preliminary test in developing a sunscreen product (active substance). The SPF values of ethanol extract and water from weighted fruit extract in sequence are 40 and 23 at a 10% concentration with ultra-category.

1. INTRODUCTION

The heat wave phenomenon in recent years has impacted increasing morbidity or health complaints, especially in older people, children, and men [1]. Asian countries also experienced extreme temperatures during April 2023, reaching 51 ºC [2]. In Indonesia, high sunlight exposure reaches 2000 kWh/m^2 per year. High temperatures and a high UV light index accompanied by a decrease in rainfall intensity in Indonesia are increasingly worsening global warming conditions [2].

UV rays are needed by living creatures as a source of energy, helping growth and metabolism processes, and helping the synthesis of vitamin D. However, the depletion of the ozone layer causes very high UV radiation, especially in equatorial areas. Skin exposed to high UV rays is darker, blackened, dull, and sunburned. In the long term, exposure to high UV rays causes black spots and accelerates skin aging, such as sagging skin and wrinkles and the risk of skin cancer [3], [4]. In addition, around 90% of damage to skin cancer is caused by exposure to UV rays. UV A rays can penetrate and damage skin cell membranes and their DNA, accelerating skin aging, wrinkles, and black spots. In contrast, UV B rays have high energy radiation, can damage DNA, suppress the skin's immune system, and are the leading cause of melanoma [5].

One effort to ward off the harmful effects of UV rays is to use sunscreen that contains SPF to absorb and reflect sunlight. The greater the SPF value, the higher the protection against the skin. SPF is a number that refers to the level of sunscreen protection against sunlight. The SPF number refers to how long the body's skin can stay in the sun without experiencing sunburn when using sunscreen [6]. Sunscreen with a wide range of protection (covering UV, visible, and infrared rays) is preferred because it reflects UV radiation and has been proven to reduce skin damage [7].

Sunscreen is formulated for all activities and all skin types with specific active ingredients, such as oxybenzone, paraben, titanium dioxide, zinc oxide, and homosalate. However, these chemicals are reported to have side effects such as photoallergic, dermatitis, and urticaria [8]. On the other hand, natural active ingredients are in great demand because they contain high levels of vitamins and minerals, antioxidants, and anti-inflammatories, which are healthy for the skin. It is also abundantly available, accessible to obtain, and environmentally friendly. Several plant extracts have been used as active ingredients in sunscreen, including aloe vera, tomato, pomegranate, green tea, cucumber, grapes, almonds, African tulip tree, saffron, shea butter, jojoba oil, carrot seed oil, and soybean oil [9].

A typical Central Kalimantan plant, the sour eggplant (*Solanum ferox*) fruit, has been researched to have high antioxidant content. This fruit is known as sour eggplant which is widely used by Dayak people as a vegetable. *Solanum ferox* is also used for fever, cough, asthma, body aches, loss of appetite, sore throat, wound medicine, syphilis, and even sexual disorders in women for worm infections and skin diseases. The antioxidant test of the ethanol extract of *Solanum ferox* fruit using DPPH showed an IC50 value of 177.16 ppm [10]. It is also effective in suppressing the growth of *Pseudomonas sp* bacteria and reducing pathogenic bacteria in the fish's body. Meanwhile, the ethanol extract of *Solanum ferox* roots shows activity against *Aeromonas hydrophila* and *Pseudomonas fluorescens* [11]. The high antioxidant content correlates with the SPF value, thus opening the potential of *Solanum ferox* as an active ingredient in sunscreen formulations.

2. EXPERIMENTAL METHODS

2.1. Materials

The materials used were sour eggplant (*Solanum ferox)* fruit, distilled water, filter paper, and chemical reagents with pro analysis grade from Merck (ethanol, ethyl acetate, Meyer's reagent, Dragendorff's reagent, concentrated H_2SO_4, concentrated HCl, Mg powder, $FeCl_3$, and acetic acid anhydride).

2.2. Instrumentations

The instruments used are PRIO Evaporator Horizontal Rotary, PRIO water bath digital, UV-Vis spectrophotometer Shimadzu UV-1700 variant, and FTIR spectrophotometer Shimadzu 8400s variant.

2.3. Procedure

2.3.1. Extraction of *Solanum ferox* fruits

Fresh and well-ripe *Solanum ferox* fruit were selected, washed clean, and peeled to take the flesh. As much as 500 grams of *Solanum ferox* fruit flesh was mashed using a blender and macerated with 96% ethanol solvent every 24 hours for 3x24 hours [12]. The filtrate was separated and concentrated until a solid gel texture was obtained, and the % yield was calculated. Then, the concentrated ethanol extract of *Solanum ferox* fruits is fractionated using water as a solvent. The soluble extract was called the water fraction, and the rest was called the ethanol fraction.

2.3.2. Phytochemical Test

2.3.3.1 Alkaloid Test

A total of 1 mL of ethanol extract was put into a test tube, and then a few drops of Meyer and Dragendorff reagent were added to each. The formation of a white, orange, or brown precipitate indicates positive alkaloid test results [12].

2.3.3.2 Flavonoid Test

The flavonoid test was carried out using the Tatang method. As much as 1 mL of ethanol extract, Mg metal powder, and three drops of concentrated HCl were added. The formation of an orange-red color indicates a positive test for the flavonoid test [12].

2.3.3.3 Phenolic Test

Ethanol extract (1 mL) was put into a test tube, and iron (III) chloride ($FeCl_3$) was added. The presence of phenolic compounds is indicated by the formation of intense blue, green, red, purple, and black colors [12].

2.3.3.4 Steroid and Terpenoid Test

A total of 5 drops of ethanol extract were dropped into the two holes of the drip plate and left until dry. Then, each added three drops of concentrated H_2SO_4 for the terpenoid test, one drop of acetic acid anhydride, and one drop of concentrated H_2SO_4 in the other hole for the steroid test. The formation of a blue or green color indicates the presence of steroids, and if a red or purple color forms, it means the presence of terpenoids [12].

2.3.3.5 Saponin Test

Ethanol extract (1 mL) was put into a test tube and shaken vigorously. Positive results for the presence of saponin are indicated by the formation of stable/permanent foam for ± 15 minutes[12].

2.3.3. Identification of Secondary Metabolites

Each fraction of *Solanum ferox* fruit extract was filtered using a membrane filter. Then, the samples were analyzed with a Shimadzu 8400s variant of the FTIR spectrophotometer.

2.3.4. SPF Values Determination

Determination of the SPF value is carried out using the spectrophotometric method using dilution with the calculation of the SPF value referring to the Mansur method.

$$SPF = CFx \sum_{290}^{320} \quad EE(\lambda)xI(\lambda)xAbs(\lambda) \qquad (1)$$

Information:
CF : Correction factor (=10)
EE : Erythemal effect spectrum
I : UV intensity spectrum
Abs : Absorbance of sunscreen product

TABEL I. EE x I standard values in determining SPF values [13].

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

3. RESULTS AND DISCUSSION

3.1. Extraction of Extraction of Solanum ferox fruits

This research was carried out using a maceration method for 3x24 hours, then filtered to obtain an ethanol extract. The extract was evaporated to concentrate the sample by removing the solvent to obtain a thick ethanol extract [14]. This extract has a dark brown solid-gel texture, as shown in Figure 1. The percentage yield of the extract shown in Table II is 5.5347%.

TABEL II. Yield of ethanol extract of *Solanum ferox* fruits.

Simplicia (gram)	A solid-gel extract ethanol (gram)	% Yield
500	27.6736	5.5347

3.2. Phytochemical Test

TABEL III. Phytochemical test of *Solanum ferox* fruit.

No	Type of group test	Reagents	Results
1	Steroids	Acetic acid anhydride + One drop of concentrated H_2SO_4	(-)
2	Terpenoids	Concentrated H_2SO_4	(+)
3	Alkaloids	Meyer and Dragendorff's reagent	(+)
4	Flavonoids	Mg powder + concentrated HCl	(+)
5	Saponin		no foam
6	Phenolics	Iron (III) chloride ($FeCl_3$)	(+)

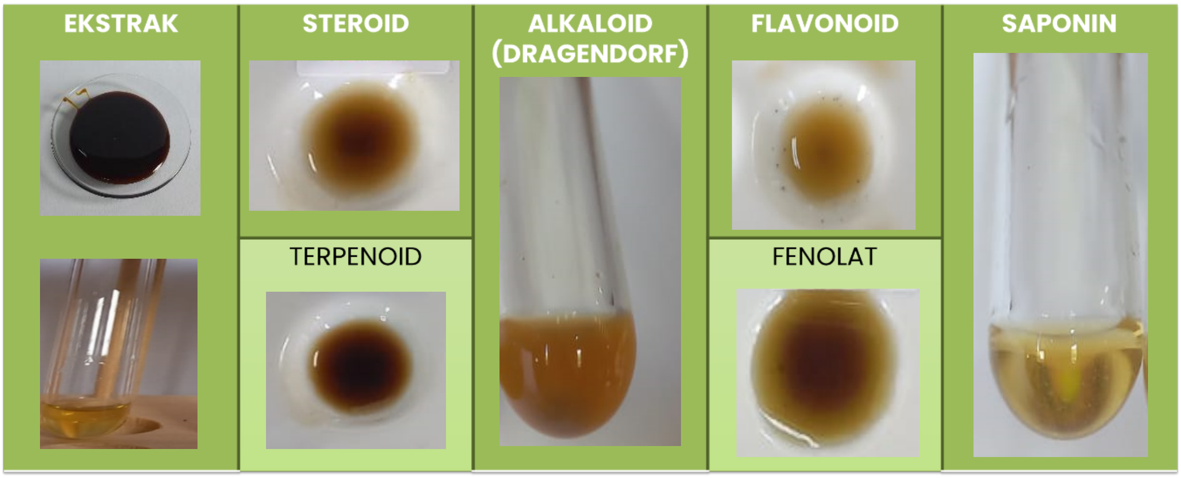


Figure 1. Phytochemical test of *Solanum ferox* fruits.

Table III and Figure 1 show the phytochemical test of the ethanol extract of *Solanum ferox* fruits. Based on Table III, the terpenoid compound class in the phytochemical test is characterized by a brick-red color change as a positive test after the addition of concentrated H_2SO_4. The presence of alkaloid compounds in the ethanol extract of *Solanum ferox* fruits was detected as an orange color change after adding the Dragendorff reagent. Flavonoid and phenolic compounds were also detected with an orange-red color change after adding Mg metal powder and concentrated HCl (flavonoids) and a blackish-red color after adding $FeCl_3$. It indicates terpenoid, alkaloid, flavonoid, and phenolic compounds dominate the ethanol extract. These results are in line with Syarpin *et al.* [10] regarding the content of flavonoid, alkaloid, and terpenoid compounds in the ethanol extract of sour eggplant fruit. The methanol extract from its leaf and fruit also showed similar compound content in phytochemical screening [15], [16].

3.3. Identification of Secondary Metabolites

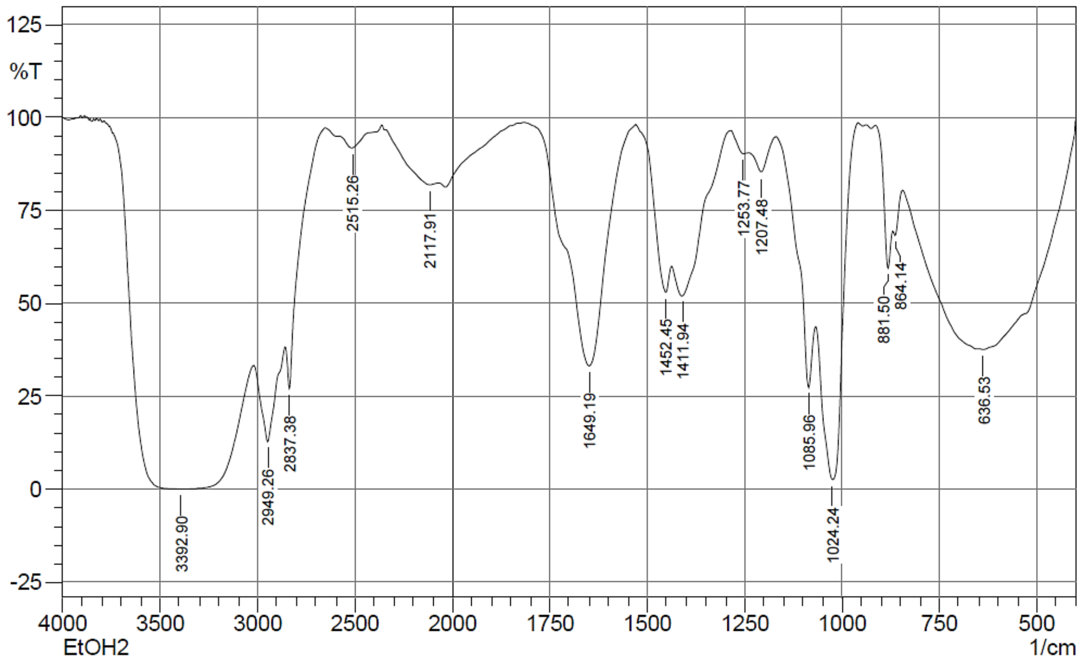


Figure 2. The IR spectrum of ethanol extract of *Solanum ferox* fruit.

Figure 2 shows the IR spectrum data of ethanol extract of *Solanum ferox* fruit, which shows a broad absorption band at wave number 3392 cm^{-1} as vibrations from the OH group. This is supported by the absorption of a single C-O group (1163 cm^{-1}). Absorption at wave number 1649 cm^{-1} indicates the (C=C) olefin group. Absorptions of 2949 and 2837 cm^{-1} indicate aliphatic C-H groups, which are supported by the absorption of CH_2 (1452 cm^{-1}) and CH3 (1411 cm^{-1}) groups. Absorption at a wave number of 1024 cm^{-1} indicates the presence of C-O ether [14], [17]. Based on the results of the interpretation of FTIR data from the ethanol extract, it was found that the group of compounds in the extract were flavonoids, which had a positive correlation with the phytochemical tests obtained.

3.4. SPF Values Determination

TABLE IV. SPF values of the Solanum ferox fruits ethanol extract.

Solvent Fractions	Concentration of Fraction (%v/v)	SPF values	Types of sun protection
Water fractions (ρ)	10	23.06	Ultra
	8	15.42	Ultra
	6	12.60	Ultra
	5	11.60	Ultra
	4	9.06	Ultra
Ethanol fractions	10	40.00	Ultra
	8	28.32	Ultra
	6	28.95	Ultra
	5	24.28	Ultra
	4	23.42	Ultra

Table IV shows the SPF values and types of protection of *Solanum ferox* fruit extract. *Solanum ferox* fruit extract has excellent potential as an SPF agent, with the best results being in the ethanol fraction with an SPF value of 40 for an extract concentration of 10%. Then, the best sunscreen activity test results (SPF value) for the water fraction at the same concentration of 10% obtained a result of 23.06. The SPF value is almost equivalent to the ethanol fraction sunscreen activity test results at the low concentration tested (4%). Based on the level of protection against sunlight, *Solanum ferox* fruit extract with ethanol and water fractions is categorized in the ultra-protection for all concentration ranges tested (4-10%). Thus, the sunscreen activity of the ethanol fraction is higher than the water fraction. In that case, *Solanum ferox* fruit extract offers excellent potential as an alternative ingredient for protection from sunlight.

Similar research on natural ingredient extracts with SPF values has been explored in recent years. Salsabila *et al.* (2021) used guava leaf ethanol extract at 10.14 % concentration and showed an SPF value of 35,56 in the ultra-protection category [18]. Other research showed that red-fleshed pitaya peel ethanol extract has an SPF value of 35.02 at a concentration of 23.76% [19]. Based on the result, the ethanol fraction of *Solanum ferox* fruit has a more excellent SPF agent than guava leaf and red-fleshed pitaya peel ethanol extract. Several plant extracts, such as aloe vera, tomatoes, pomegranates, green tea, cucumber, grapes, and almonds, have been studied to protect against UV rays and are also used in skincare formulations [9].

Sunscreens, as a skincare product, combine several ingredients that protect the skin by absorbing, blocking, or scattering UV radiation. Based on the wavelength, there are three types of UV rays: UVA (315-400 nm), UVB (290-315 nm), and UVC (200-290 nm). Figures 3-4 provide the spectra of UV ray absorption of *Solanum ferox* fruit extract in various solvent extraction and concentrations. The results show that Solanum ferox fruit extract strongly protects against UV by absorbing UV radiation, especially UV A and UV B rays. Both combination protection of UVA and UVB rays as a sunscreen formulation is more efficient in protecting the skin than alone. Therefore, *Solanum ferox* fruit extract can be considered an alternative ingredient for sunscreen formulation.

Figure 3 shows the comparative spectra of UV ray absorption of the water and ethanol fractions with different concentrations at the same SPF values of 23. It shows that the ethanol fraction provides better sunscreen protection than the water fraction, even in smaller concentrations. Moreover, the ethanol fraction offers the best UV absorption in the UV B area. Based on Table I, the highest consideration for sunscreen activity is the sunscreen's ability to absorb UV B rays, especially in 300, 305, and 310 nm, which have the highest EE x I value.

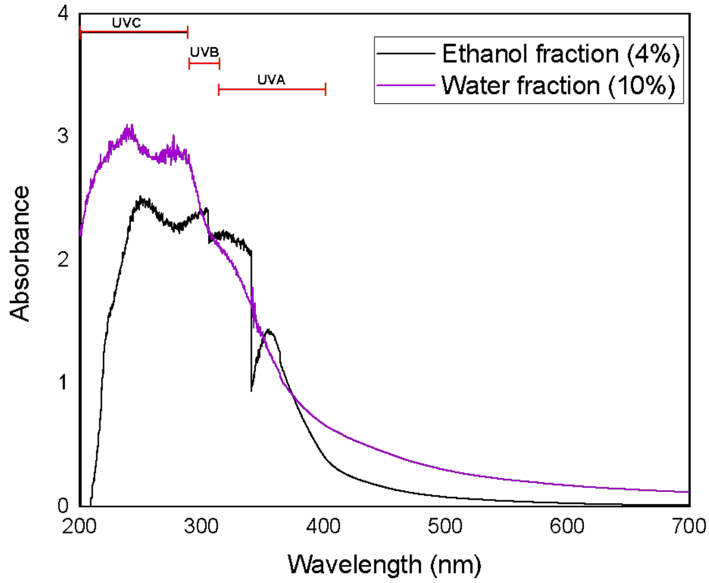


Figure 3. Absorption spectra of air and ethanol fraction extracts of *Solanum ferox* fruits at the same SPF value =~23.

Figure 4 shows the UV absorption spectra of the ethanol and water fractions at the lower concentration tested (4%). Both fractions have optimum absorption in UV C, and the absorption intensity decreases along with higher wavelengths in UV B and UV A. In the water fraction, the absorption intensity is UVC>UVB>UVA, while in the ethanol fraction, the absorption intensity is UVC≈UVB>UVA. Overall, it is observed that the UV absorption of the ethanol fraction is higher than the water fraction in almost all ranges of UV A, UV B, and UV C at the same concentration. In other words, the ethanol fraction partially absorbs UV rays C and the whole range of UV A and UV B more effectively than the water fraction. For this reason, based on the SPF value (Table IV), the ethanol fraction has higher sunscreen activity than the water fraction at the same concentration.

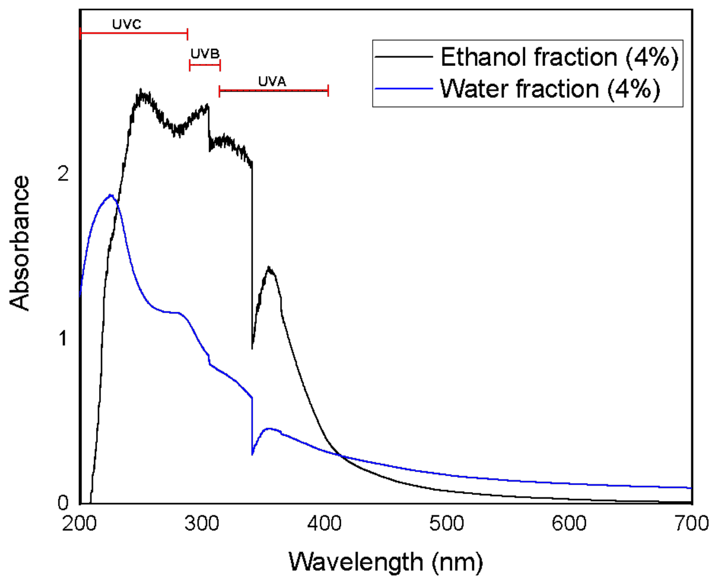


Figure 4. Absorption spectra of air and ethanol fraction extracts of *Solanum ferox* fruits at the same concentration of 4%.

Figure 5 shows the ethanol fraction can still absorb UV rays even at dilute concentrations (0.4%). The more dilute the concentration of the ethanol fraction used, the more the absorption intensity decreases significantly in the UV B absorption, and the absorption shifts towards shorter wavelengths (UV C) resembling the water fraction with optimum absorption in the UV C (UVC>UVB>UVA) in Figure 4. Therefore, it is best to use a concentrated ethanol fraction (4% or higher) for a sun protection agent that provides higher UV B absorption or insignificant differences in UV B and UV C absorption.

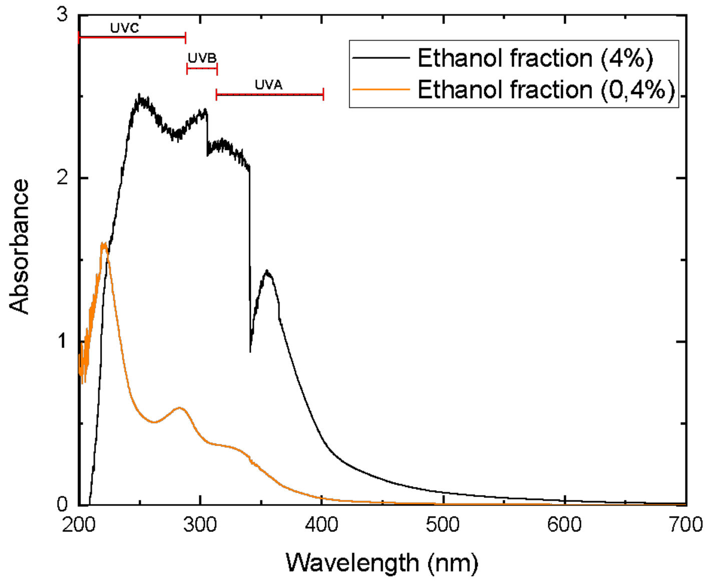


Figure 5. Absorption spectra of ethanol fraction of *Solanum ferox* fruits at concentrations 4% and 0,4%.

Compared to UV C rays, which are entirely absorbed by the ozone layer and atmosphere, UV A rays are hardly absorbed. Even though UV A rays have low energy, approximately 95% of UV A rays are not blocked by the ozone layer and reach the earth's surface, penetrating through clouds and glass. These rays can induce the production of reactive oxygen species (ROS), damage blood vessels, collagen fibers, and elastic fibers, and cause skin aging. On the contrary, UV B rays have high energy; around 5-10% can reach the earth's surface and cause skin redness. Thus, most UV radiation that reaches Earth is UV A and UB rays, and it is essential to seek a sunscreen formulation with both UV B and UV A absorption to give higher protection. The previous study showed that daily protection can reduce lifetime exposure by 50% or more and even low-level (SPF 4–10) sunscreens can give significant benefits [5].

4. CONCLUSIONS

Sour eggplant (*Solanum ferox*) fruit has the highest SPF value of 40 in the ethanol fraction and 23 in the water fraction for an extract concentration of 10% in both fractions. Based on the level of protection against sunlight, both *Solanum ferox* fruit fractions are ultra-protection categories in all concentration ranges tested (4-10%). Therefore, *Solanum ferox* extract can be used as an alternative ingredient for protection from sunlight. The phytochemical tests that have been carried out show that the ethanol extract positively contains terpenoid, alkaloid, flavonoid, and phenolic compounds, which are supported by the presence of OH, C-O, C=C olefin, C-O ether, and aliphatic CH groups (CH_2 and CH_3) in the IR spectrum.

Acknowledgment

Thank you to LPPM Palangka Raya University for funding this research through DIPA PNBP LPPM UPR 2023 and the Chemistry Laboratory, Palangka Raya University, as the facilitator for the extraction and phytochemical testing of *Solanum ferox* ethanol extract. Thanks to Ujung Pandang State Polytechnic (PNUP) for FTIR analysis.

References

[1] M. Li, S. Gu, P. Bi, J. Yang, and Q. Liu, "Heat waves and morbidity: Current knowledge and further direction-a comprehensive literature review," *International Journal of Environmental Research and Public Health*, vol. 12, no. 5. MDPI, pp. 5256–5283, May 18, 2015. doi: 10.3390/ijerph120505256.

[2] A. Yananto and R. M. Sibarani, "Analisis Kejadian El Nino dan Pengaruhnya terhadap Intensitas HUjan di Wilayah Jabodetabek (Studi Kasus : Periode Puncak Musim Hujan Tahun 2015/2016)," *Jurnal Sains dan Teknologi Modifikasi Cuaca*, vol. 17, no. 2, pp. 65–73, 2016.

[3] R. Dummer and T. Maier, "UV Protection and Skin Cancer," *Recent Results in Cancer Research*, vol. 160, pp. 7–12, 2002.

[4] C. Ulrich *et al.*, "Prevention of non-melanoma skin cancer in organ transplant patients by regular use of a sunscreen: A 24 months, prospective, case-control study," *British Journal of Dermatology*, vol. 161, no. SUPPL. 3, pp. 78–84, Nov. 2009, doi: 10.1111/j.1365-2133.2009.09453.x.

[5] G. Nole and A. W. Johnson, "An analysis of cumulative lifetime solar ultraviolet radiation exposure and the benefits of daily sun protection," *Dermatol Ther*, vol. 17, pp. 57–62, 2004.

[6] G. J. Nohynek and H. Schaefer, "Benefit and risk of organic ultraviolet filters," *Regulatory Toxicology and Pharmacology*, vol. 33, no. 3, pp. 285–299, 2001, doi: 10.1006/rtph.2001.1476.

[7] S. Seité and A. M. A. Fourtanier, "The benefit of daily photoprotection," *J Am Acad Dermatol*, vol. 58, no. 5 SUPPL. 2, May 2008, doi: 10.1016/j.jaad.2007.04.036.

[8] J. C. DiNardo and C. A. Downs, "Dermatological and environmental toxicological impact of the sunscreen ingredient oxybenzone/benzophenone-3," *Journal of Cosmetic Dermatology*, vol. 17, no. 1. Blackwell Publishing Ltd, pp. 15–19, Feb. 01, 2018. doi: 10.1111/jocd.12449.

[9] P. Kantivan Goswami, M. Samant, R. Srivastava, and P. K. Goswami, "Natural Sunscreen Agents: A Review," *Scholars Academic Journal of Pharmacy*, vol. 2, no. 6, pp. 458–463, 2013, [Online]. Available: www.saspublisher.com458

[10] S. Syarpin, W. Nugroho, and S. Rahayu, "Uji Fitokimia dan Antioksidan Ekstrak Etanol Buah Terung Asam (Solanum ferox L) Phytochemistry Screening and Antioxidant Test of Ethanol Extract of Terung Asam (Solanum ferox L)," *Accta Pharmaciae Indonesia*, vol. 6, no. 2, pp. 46–50, 2018, doi: 10.5281/zenodo.3707211.

[11] E. H. Hardi, R. A. Nugroho, I. W. Kusuma, W. Suwinarti, A. Sudaryono, and R. Rostika, "Borneo herbal plant extracts as a natural medication for prophylaxis and treatment of Aeromonas hydrophila and Pseudomonas fluorescens infection in tilapia (Oreochromis niloticus)," *F1000Res*, vol. 7, p. 1847, Nov. 2018, doi: 10.12688/f1000research.16902.1.

[12] T. Shabur Julianto, *Fitokimia Tinjauan Metabolit Sekunder dan Skrining Fitokimia*, 1st ed. Yogyakarta: Universitas Islam Indonesia, 2019.

[13] C. D. Kaur and S. Saraf, "In vitro sun protection factor determination of herbal oils used in cosmetics," *Pharmacognosy Res*, vol. 2, no. 1, pp. 22–25, Jan. 2010, doi: 10.4103/0974-8490.60586.

[14] S. Y. Kalalinggi, N. H. Soekamto, and F. Firdaus, "Functional Group Identification and Toxicity of Piper crocatum Red Betel Acetone Extract against Artemia salina by Brine Shrimp Lethality Test Method," *IJCA (Indonesian Journal of Chemical Analysis)*, vol. 6, no. 2, pp. 133–143, Sep. 2023, doi: 10.20885/ijca.vol6.iss2.art5.

[15] H. Hazimah, Z. Azharman, V. Rahyuti, and A. Afriliani, "Aktivitas Antibakteria Ekstrak Metanol Daun Tanaman Solanum ferox L," *Jurnal Rekayasa Sistem Industri*, vol. 4, no. 1, pp. 2621–1262, 2018.

[16] W. Nugroho, L. Rosmainar, and E. P. Toepak, "Skrining Fitokimia dan Uji Antioksidan Menggunakan Metode DPPH pada Ekstrak Metanol Buah Terung Asam Besar (Solanum Ferox Linn)," 2023.

[17] D. L. Pavia, G. M. Lampman, and G. S. Kriz, *Introduction to Spectroscopy*, 3rd ed. Washington: Thomson Learning, Inc., 2001.

[18] S. Salsabila, I. Rahmiyani, and D. S. Zustika, "Nilai Sun Protection Factor (SPF) pada Sediaan Lotion Ekstrak Etanol Daun Jambu Air (Syzygium aqueum)," *Majalah Farmasetika*, vol. 6, no. 1, pp. 123–132, 2021, doi: https://doi.org/10.24198/mfarmasetika.v6i0.

[19] R. Vijayakumar, S. S. Abd Gani, U. H. Zaidan, M. I. E. Halmi, T. Karunakaran, and M. R. Hamdan, "Exploring the Potential Use of Hylocereus polyrhizus Peels as a Source of Cosmeceutical Sunscreen Agent for Its Antioxidant and Photoprotective Properties," *Evidence-based Complementary and Alternative Medicine*, vol. 2020, pp. 1–12, 2020, doi: 10.1155/2020/7520736.