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Evaluation Of Sun Protection Factor Value Of Water, Ethyl Acetate, And N-Hexane Fractions Of Ethanolic Extract Of Crystal Guava Fruit (Psidium Guajava. L.) By UV-VIS Spectrophotometry

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Abstract

Excessive exposure to ultraviolet (UV) rays can cause various skin damage, so an effective and safe sunscreen preparation is needed. Natural ingredients, especially crystal guava fruit (Psidium guajava L.), are known to contain secondary metabolite compounds such as flavonoids that have the potential as natural photoprotective agents. Crystal Guava Fruit (Psidium guajava L.) contains several secondary metabolite compounds, namely flavonoids, saponins, and terpenoids. The content of secondary metabolites plays a role as a Sun Protection Factor, because these compounds are able to absorb UV A and UV B rays well. This study aims to determine the Sun Protection Factor (SPF) value of ethyl acetate, n-Hexane, and water fractions from crystal guava fruit (Psidium guajava L.) in vitro using the UV-Vis spectrophotometry method and to determine the fraction with the best sunscreen activity. The extract was prepared by maceration using 96% ethanol solvent, then the extract was fractionated in stages using n-Hexane, ethyl acetate, and water. SPF values were determined in vitro using UV-Vis spectrophotometry at a wavelength of 290-320 nm. Each fraction was prepared in several concentrations: 200, 400, 600, 800, and 1000 ppm. The study showed that all fractions had sunscreen activity with SPF values that increased with increasing concentration. The ethyl acetate fraction showed the highest activity with an SPF value of 24.249 at a concentration of 1000 ppm which is included in the ultra protection category. This study shows that the water, ethyl acetate, and n-Hexane fraction of crystal guava fruit (Psidium guajava L.) have the potential as SPF because they are in the range 2-4 (minimum category).

Keywords: Fractionation, Psidium Guajava L., SPF, UV-Vis Spectrophotometry.

INTRODUCTION

As a tropical country on the equator, Indonesia receives year-round sun exposure with high UV intensity. In 2021, the UV index in Indonesia reached the very dangerous category (9–12). UV rays are divided into three types based on wavelength: UV-A (320–400 nm), UV-B (290–320 nm), and UV-C (100–290 nm). Excessive exposure to UV rays can cause various skin problems such as pigmentation, sunburn, premature aging, and skin cancer (Damayanti et al., 2024). UV exposure increases melanin production by 20–30% in exposed skin (Narayan et al., 2020). WHO (2020) reported more than 15 million cases of skin cancer due to UV exposure. The risk of melanoma increases 2–3-fold in individuals with high UV exposure. Skin cancer is also triggered by mutations in the p53 gene (Armstrong & Kicker, 2020). Sunburn occurs when the erythema dose reaches 20–50 mJ/cm², and intense UV-B exposure of more than 20 minutes can increase redness 2–4-fold (Pathak, 2021). UV exposure also accelerates skin aging by increasing dermal elastosis by up to 40%, decreasing collagen synthesis by 40%, and increasing the wrinkle index by 20% (Wang et al., 2020). In the past two decades, the global scientific discourse has fundamentally shifted from mere efficacy to the safety and sustainability of photoprotective products. A study by Tang et al. (2024) confirmed that UV exposure is not only beneficial in vitamin D synthesis but also a full-fledged physical carcinogen requiring comprehensive mitigation. Food and Drug Administrations in various countries have begun to tighten regulations on the use of synthetic chemicals such as oxybenzone and octinoxate, which have been shown to have potential endocrine disruption and toxicity to aquatic ecosystems. This situation has triggered a shift in the research paradigm from the development of synthetic UV filters to the exploration of natural materials that are more environmentally friendly and have a superior safety profile. Indonesia as a mega-biodiversity country has a strategic position in the

development of active photoprotective ingredients based on traditional medicinal plants that have not been optimally explored.

The scientific perspective linking antioxidant capacity with photoprotective activity has become the foundation for exploring natural ingredients for the development of plant-based sunscreens. Phenolic and flavonoid compounds, in addition to their ability to absorb ultraviolet radiation at specific wavelengths, also act as antioxidants that neutralize free radicals induced by UV exposure. Therefore, a review of previous research focused specifically on quantitative data on the IC50 antioxidant values and total flavonoid content of guava (Psidium guajava L.) fruit extracts and fractions, which served as the primary basis for conducting SPF evaluation studies on crystal guava fruit fractions.

The most relevant study, serving as a direct reference for this research, was conducted by Larasati et al. (2023), who tested the antioxidant activity of a 70% ethanol extract of Australian guava (Psidium guajava L.) using the DPPH method via UV-Vis spectrophotometry. The results showed that it was categorized as very potent with a low IC50 value of 0.784 µg/mL; a lower IC50 value indicates a higher antioxidant value. Subsequent research (Lutfiah et al., 2025) found that guava (P. guajava) fruit extract contained flavonoids at 24.3850 mg QE per gram of extract. Research (Widiyati et al., 2023) indicated that the sunscreen effectiveness of guava (Psidium guajava L.) leaf ethanol extract had an SPF of 6.765, which is considered low at a concentration of 200 ppm. Phytochemical screening revealed that crystal guava contains secondary metabolites such as antioxidants (flavonoids), anti-inflammatory compounds (terpenoids and steroids), tannins, alkaloids, and polyphenols (Wardani et al., 2024).

Based on research by Wardani et al. (2024), ethyl acetate is a solvent that is easily evaporated, non-hygroscopic, has low toxicity, and is semi-polar, so it is expected to extract both polar and non-polar compounds from the sample. Compounds that can be extracted include polyphenols and flavonoids (Sandrasari et al. 2023). Previous research on determining the Sun Protection Factor (SPF) value of guava fruit ethanol extract showed a result of 46.183, categorized as ultra protection at a concentration of 1000 ppm (Zulkarnain et al. 2024).

Based on previous research, sunscreen activity tests have been conducted on guava fruit flesh (Psidium guajava L.). Guava fruit has the potential to be developed into a natural Sun Protection Factor, based on previous research, sunscreen activity tests have been conducted on guava fruit flesh (Psidium guajava L.), no one has studied the determination of the Sun Protection Factor value of the Ethyl Acetate Fraction of Crystal Guava Fruit. Therefore, researchers are interested in making water, ethyl acetate, and n-Hexane fractions of crystal guava fruit and then determining the Sun Protection Factor value using the UV-Vis spectrophotometry method.

RESEARCH METHODS

The method used in this study is an experimental method. This study aims to determine the Sun Protection Factor value contained in the fraction of ethyl acetate, n-hexane, and guava fruit water (Psidium guajava L.) using UV-Vis spectrophotometry. The research stages begin with sampling, plant determination, preparation of simple drugs, preparation of extracts, preparation of fractions, phytochemical screening, preparation of test solutions, testing of Sun Protection Factor values, and data analysis.

Data analysis

Calculation of Sun Protection Factor (SPF) values. The SPF values of ethyl acetate, n-hexane, and guava fruit water fractions at each test concentration were calculated using the Mansur method (Marliana et al., 2022) as follows:

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

In this case,
CF = Correction factor 10
EE = Erythremal Effect
I = Intensity
A = Absorbansi

The EE x I value is a constant and can be found in previous literature. A is the absorbance of the extract solution after obtaining the SPF (Sun Protection Factor) value. The extract's protective ability is categorized based on the literature (Marliana et al., 2022).

In analyzing the research data, the maximum wavelength of the crystal guava fruit was determined using a UV-Vis spectrophotometer. After obtaining the absorbance for each concentration, the SPF (Sun Protection Factor) value of the crystal guava fruit fraction was calculated, with the sunscreen protection category value created in a table using Microsoft Excel.

RESULTS AND DISCUSSION

The initial stage in this research is the collection of raw materials of crystal guava, which will then be determined by the plant to guarantee the authenticity of the research material and ensure the identity of the plant from the sample used. This determination aims to ensure the accuracy of the sample used in the research clearly from the plant used so as to reduce the possibility of errors (Krismayadi et al., 2024). This research uses crystal guava fruit obtained from Gemawang Village, Dukuh, Ngargoyoso District, Karanganyar Regency, Central Java. The sample was then determined at the Center for Research and Development of Medicinal Plants and Traditional Medicines Tawangmangu (UPF Yankestard) on Jl. Raya Lawu No.11. Tawangmangu, Kalisoro, Karanganyar Regency, Central Java Province to find out and ensure the plant species to be studied are in accordance with the desired number TL.02.04 / D.XI.6 / 34484.966 / 2025. The results of the determination of the plant to be studied matched the desired target, namely the Crystal Guava plant with the Latin name Psidium guajava L.

The collection of raw crystal guava material, also obtained from Gemawang Village, Dukuh, Ngargoyoso District, Karanganyar Regency, Central Java, was carried out after confirming the plant's identity. A 5 kg sample of Crystal Guava was collected and wet sorted to remove any dirt adhering to the guava by removing unnecessary parts. Subsequently, the guava was washed to remove any remaining dirt by running clean water over it, then drained. The slicing process was then carried out to accelerate the drying process by thinly slicing it with a knife. After slicing, the sample was dried under sunlight, covered with a black cloth to prevent the decomposition of the Crystal Guava's chemical constituents (Llupitasari & Azzahra, 2025). The purpose of drying is to ensure long-lasting storage of medicinal plants. Reducing the water content prevents quality degradation or damage due to the growth of mold microorganisms (Krismayadi et al., 2024).

Medicinal plants are considered dry if they crumble easily when crushed or break easily. After the dried samples were dry sorted to remove foreign matter, unnecessary plant parts, and other impurities remaining in the dried medicinal plants, a dry sample of 268.25 grams of Crystal Guava was obtained. The dried medicinal plants were then ground into powder using a blender. Converting the medicinal plants into powder form aims to increase the efficiency of extracting active compounds during the extraction process. One factor influencing the solubility of a substance is particle size; the smaller the particle size, the larger the surface area, thus accelerating the dissolution process and increasing extraction efficiency (Arifin et al., 2025). Then the simplicia is sieved with a 60 mesh sieve to obtain a fine powder with a uniform size and stored in a dry and tightly closed container to avoid exposure to sunlight or the entry of insects (Handoyo & Pranoto, 2024).

The yield of crystal guava fruit simplicia was 5.36%. This yield is considered low, as it does not exceed 10% (Rahadyana et al., 2024). This is because crystal guava fruit has a high water content, as the higher the water content in fresh fruit, the lower the yield (Winaliani & Sari, 2024). The resulting

simplicia powder is brownish-green in color and has a distinctive crystal guava odor. Before proceeding to the extraction process, the simplicia powder requires quality standardization. Standardization of the simplicia powder quality is carried out to ensure the quality of the materials used for research and to ensure the simplicia meets the appropriate simplicia standard requirements (Ministry of Health, 2017). Standardization of the simplicia powder includes testing for water content, ash content, and drying loss.

The water content testing of crystal guava fruit simplicia (Psidium guajava L.) is carried out to reduce the water content of the simplicia to prevent rapid fungal growth. Determining the water content is crucial for establishing a maximum water content limit for a material, as high water levels in herbal remedies can foster bacterial and fungal growth (Krismayadi et al., 2024). This moisture content test utilizes a moisture balance tool. Two grams of herbal remedies are weighed in a moisture balance dish. The moisture balance is set at 105°C, then the lid is closed and the moisture content is waited for several minutes until the water content reading is displayed.

Based on the moisture content test of the crystal guava fruit herbal remedy, the average water content was 4.61%, indicating that the results meet the requirements for a good herbal remedy, which is less than 10% (Misrahanum et al., 2022). This water content is in accordance with the Indonesian Herbal Pharmacopoeia (2017), which states that a good extract should not have a water content exceeding 10%.

The drying loss test aims to determine the extent of compound loss during the drying process due to heating (Aziz et al., 2024). Determining drying loss is a method for measuring the remaining substance content after a sample is heated at 105°C for half an hour, causing water and several other more volatile compounds to be lost from the sample. The purpose of this test is to determine the maximum limit of substance loss during the drying process. Drying loss also aims to determine the water content and volatile compounds contained in the extract (Rusmawati et al., 2021).

Based on the results of the drying shrinkage test conducted in three replications, the average drying shrinkage of crystal guava fruit was 6.43%. The results indicate that it meets the requirements of not exceeding 10% (Misrahanum et al., 2022). If the drying shrinkage exceeds 10%, it can affect the enzymatic system and cause damage caused by microorganisms. Based on the results of this study, the drying shrinkage value of crystal guava fruit did not exceed this limit, thus concluding that this crystal guava fruit meets the established quality standards. It is also stated that a good drying shrinkage standard is no more than 10% (Ministry of Health, 2017).

This ash content test is conducted to determine mineral content, both from internal and external sources, from the initial stages until the formation of the simplex (Utami et al., 2023). High ash content indicates a high level of organic contamination. Ash content measurement involves firing in a furnace at 600°C for 4 hours. The analysis results showed that the ash content of the herbal medicine averaged 2.44%. This value complies with the provisions of the Indonesian Herbal Pharmacopoeia (Ministry of Health, 2017), as the results obtained did not exceed 10%. The purpose of the ash content test is to describe the amount of inorganic minerals contained in the herbal medicine after ashing (Rusmawati et al., 2021).

The next step, after the herbal medicine meets the quality standard requirements, is the preparation of a crystal guava (Psidium guajava L.) fruit extract. Crystal guava fruit extract is made using the maceration method. Maceration is a simple extraction method that involves soaking the sample in a specific organic solvent to separate secondary metabolites (Mutripah and Badriyah, 2024). Maceration is an extraction method that involves soaking the material in a solvent suitable for the active compound, which is extracted with low or no heating (Razoki et al., 2023). The crystal guava herbal medicine powder is placed in a maceration vessel. This maceration method uses 96% ethanol as a universal solvent. Ethanol was chosen for its selectivity, non-toxicity, good absorption, and high extraction capacity, allowing it to extract non-polar, semi-polar, and polar compounds (Wendersteyt et al. 2021).

Crystal guava fruit powder was weighed at 241.586 grams and macerated by soaking the extract in 2410 mL of 96% ethanol at room temperature for 3x24 hours with repeated stirring, followed by filtration using flannel cloth and a funnel to separate the pulp and filtrate. Stirring during the maceration process has been shown to increase homogeneity and extraction efficiency (Arifin et al., 2025). The maceration residue was then re-macerated using 600 mL of 96% ethanol for 1 day, followed by further filtration.

Re-maceration was performed because after the first maceration, some bioactive compounds remained unextracted. The re-maceration process renews the solvent, increasing the concentration gradient and maximizing compound extraction, resulting in a higher total extract yield (Pebriani et al., 2021). The resulting filtrate was then stored in a tightly closed container. The results of the remaceration were combined with the results of the initial maceration, then concentrated using a rotary vacuum evaporator at 50°C to obtain a thick extract. The results obtained from this extract preparation can be seen in Table 4.5. The thick extract was used to calculate the extract yield, which is the ratio of the weight of the resulting extract to the weight of the raw material (Lupitasari & Azzahra, 2025). Based on the results obtained, the yield of the crystal guava extract was calculated, resulting in a value of 80.72%, with an extract weight of 195 grams and a crude material weight of 241.58 grams. According to Arifin et al., 2025, calculating the yield of a sample aims to determine the amount of extract obtained during the extraction process. The higher the yield, the greater the number of compounds contained in the sample (Lupitasari & Azzahra, 2025). This high yield can be caused by secondary metabolites present in the plant, the solvent used, the temperature during the extraction process, and the finer particle size of the powder which increases the contact surface area of the sample and solvent so that the diffusion of active compounds is more efficient and the yield increases, and it can also be caused by a less efficient thickening process which allows the detection of solvent residues in the extract yield (Arifin et al., 2025). The extract yield is said to be good if the result obtained is more than 10% (Rahadyana et al., 2024). This result is in accordance with the extract quality requirements based on the testing parameters that have been set above. The results obtained are also in accordance with the standards in the Indonesian Herbal Pharmacopoeia which states that the yield is not less than 10% (Ministry of Health, 2017).

As the initial step in testing extract quality standards, extract standardization is performed. Extract standardization determines material specifications based on certain parameters to achieve standard quality levels (Rahadyana et al., 2024). Several extract standardization tests were performed, including the ethanol-free test, the water content test, and the metal-free test.

This ethanol-free test was conducted to determine the presence of ethanol in the extract, ensuring a pure extract. The purpose of the ethanol-free test was to ensure there were no solvent residues remaining, as residual ethanol can interfere with subsequent testing and produce inaccurate results (Ibriyah et al., 2025). This test was performed by placing 2 mL of the thick extract in a test tube, adding 2 drops of H2SO4 and 2 drops of acetic acid, and then heating. The results of the ethanol-free test showed that the crystal guava (Psidium guajava L.) extract no longer exhibited the characteristic ester odor of ethanol, indicating that the crystal guava extract was ethanol-free (Aziz et al., 2024).

Testing the moisture content of an extract aims to determine its water content. The moisture content in an extract should be as low as possible, as too high a content can make the material susceptible to microbial growth, which can affect its properties (Krismayadi et al., 2024). The moisture content of an extract is measured using a moisture balance.

Based on the results obtained, the average water content of the condensed extract of crystal guava (Psidium guajava L.) was 9.21%. From these water content tests, it can be concluded that the water content in the crystal guava extract meets the requirements, or it can also be said that the water content test for this extract meets the established quality standards, which is ≤ 10% (Utami et al., 2020). These results also indicate that it meets the requirements of the Indonesian Herbal Pharmacopoeia, 2017, which must be less than 10%. Determining the water content in the extract aims

to provide a minimum limit or range for the amount of water content in the extract. The higher the water content, the more susceptible it is to mold and mildew growth, thereby reducing the extract's shelf life (Krismayadi et al., 2024).

This metal-free test aims to ensure safety from hazardous metal contamination in crystal guava (Psidium guajava L.) extract. According to Iryo (2024), a positive reaction in the qualitative lead (Pb) test for metal-free extract is the formation of a yellow precipitate. This change occurs due to the reaction between KCr_2O_4 and Pb, resulting in the formation of a yellow PbCrO4 mixture. If Cd is present in the sample, Cd(OH) will form, indicated by the appearance of a pink color. The results of the metal-free examination of the thick crystal guava fruit extract. The results obtained from the Pb and Cd metal-free test showed no color change in the extract sample, indicating a negative sample, meaning the sample did not contain metal contamination (Iryo et al., 2024).

After obtaining a crystal guava fruit extract that meets quality standards, phytochemical screening is performed. Phytochemical screening, or identification of chemical compounds in the sample, aims to determine the chemical compounds present in the crystal guava fruit extract (Syahputra, 2023). Identification of chemical compounds, or phytochemical screening, is carried out to determine the secondary metabolites contained in the crystal guava fruit extract. These secondary metabolites tested qualitatively are flavonoids, alkaloids, saponins, terpenoids, and tannins. Phytochemical screening can be performed using two methods: the test tube test (color complex) and the TLC (Thin Layer Chromatography) test.

Test tube testing is a simple, rapid, and economical qualitative method that does not require complex equipment. It is therefore highly suitable for initial screening of secondary metabolites in extracts before proceeding to further testing (Pasaribu et al., 2025). The test tube test provides an initial indication of the presence of these compounds in the extract, making it important as a basis for subsequent activity testing. Based on the test tube test in the phytochemical screening of crystal guava fruit extract, it was determined that the phytochemical compounds contained within it were flavonoids, saponins, and teponoids.

This test tube test yielded a positive result for flavonoids, indicating a color change to red. This color change is due to the addition of Mg powder and HCl, which work to reduce the benzopyrone nucleus present in the flavonoid structure, forming a red to orange flavyllium salt (Kurnianto et al., 2021).

Saponins are glycoside compounds that exhibit soap-like properties when dissolved in water. They are complex, high-molecular-weight glycosides produced primarily by several plants. Their triterpene/sterol structure has a hydrophilic (sugar) and lipophilic (terpenoid aglycone) portion; these two parts cause the saponin to lower the surface tension of water when shaken vigorously, forming a stable foam (Ulandari et al., 2024). The study found a positive result for saponins, indicated by the formation of a stable foam.

Terpenoids in the test tube are affected by the addition of chloroform and concentrated sulfuric acid, resulting in an oxidation reaction and the formation of a complex color (Yana et al., 2025). A positive test for terpenoids is indicated by the formation of an orange or purple color. The test tube test for terpenoids yielded a positive result with the formation of an orange color.

The next phytochemical screening test used Thin Layer Chromatography (TLC). In the TLC test, one plate of activated silica gel was used as the stationary phase. The mobile phase used in this study was methanol and ethyl acetate in a 4:1 ratio. The TLC test yielded an Rf value of 0.64, which meets the requirements for a good Rf value, which is between 0.2 and 0.8, indicating activity. To obtain a good Rf value, the solvent is present (Rugayyah Alyidrus et al., 2022).

After conducting the phytochemical screening test on crystal guava (Psidium guajava L.), fractionation was performed. Fractionation is a process of separating compounds based on two immiscible solvents (Weliyanto et al., 2025). Fractionation is performed by dissolving 10 grams of extract in 100 ml of water and stirring until dissolved. Fractionation uses a liquid-liquid method using a separating funnel. The purpose of fractionation is to separate groups of compounds based on their

differing levels of polarity (Weliyanto et al., 2025). Fractionation of crystal guava fruit using water, n-hexane, and ethyl acetate as solvents. In the first fractionation stage, 100 ml of n-hexane was added. After the n-hexane was added, the mixture was shaken and allowed to stand for a while until two phases formed: the upper phase being the n-hexane phase and the lower phase being the water phase. The fractionation was repeated twice. The n-hexane fractionation results were pooled together.

Subsequent fractionation used ethyl acetate as solvent. Ethyl acetate has semi-polar solvent properties, a solvent used to dissolve flavonoids, tannins, and alkaloids (Wardani et al., 2024). This ethyl acetate fractionation is the same as that carried out on n-hexane, after adding 100 ml of ethyl acetate solvent, it is shaken and left to form two phases, the upper phase is ethyl acetate because the specific gravity of ethyl is smaller than the water phase and the lower phase is the water phase. The fractionation results from the two phases are separated and the water phase fractionation is repeated twice. The results of the two separated fractions are collected according to their phases.

Based on the results in the table above, the yield of the n-hexane fraction was 17.04%, the ethyl acetate fraction was 55.03%, and the water fraction was 65.55%. This is because this test uses a liquid-liquid method with a separating funnel, which aims to separate compounds with different polarities (Soemari et al., 2024). This is what causes the water fraction to have a higher yield value compared to the ethyl acetate and n-hexane fractions. This is because water itself is polar, so it contains many compounds such as sugars, glycosides, alkaloids, tannins, saponins, and terpenoids. In contrast, the ethyl acetate fraction is semi-polar and the n-hexane fraction is non-polar, so the compounds contained therein are only based on their polarity, such as flavonoids, phenolics, several alkaloids, and certain terpenoids found in the ethyl acetate fraction and compounds found in the n-hexane fraction such as fats and oils, as well as steroids and non-polar terpenes (Nurdjananah et al., 2023).

The scientific perspective linking antioxidant capacity with photoprotective activity has become the basic foundation in the exploration of natural ingredients for the development of plant-based sunscreens. Phenolic and flavonoid compounds, in addition to their ability to absorb ultraviolet radiation at specific wavelengths, also act as antioxidants that neutralize free radicals induced by UV exposure (Fitriansyah et al., 2025). Therefore, this review of previous research focused specifically on quantitative data on the IC50 antioxidant values and total flavonoid content of guava (Psidium guajava L.) fruit extracts and fractions, which served as the primary basis for the SPF evaluation study on crystal guava fruit fractions.

The most relevant study, serving as a direct reference for this research, was conducted by Larasati et al., 2023, who tested the antioxidant activity of a 70% ethanol extract of Australian guava (Psidium guajava L.) using the DPPH method via UV-Vis spectrophotometry. The results showed that it was categorized as very potent with a low IC50 value of 0.784 µg/mL. The lower the IC50 value, the higher the antioxidant value. The higher the antioxidant value, the higher the resulting SPF value. The following study (Lutfiah et al., 2025) found that the flavonoid content in guava fruit extract (P. guajava) was 24.3850 mg QE per gram of extract. Research (Widiyati et al., 2023) stated that the effectiveness of sunscreen in guava leaf ethanol extract (Psidium guajava L.) had an SPF value of 6.765, which is included in the low category at a concentration of 200 ppm of ethanol extract. Phytochemical screening showed that crystal guava plants contain secondary metabolites such as antioxidants (flavonoids), anti-inflammatory compounds (terpenoids and steroids), tannins, alkaloids, and polyphenols (Wardani et al., 2024).

The initial stage in testing the SPF value of the crystal guava fruit fraction was conducted using a UV-Vis spectrophotometer, an analytical instrument that operates based on the principle of absorption of ultraviolet and visible light by chemical compounds in solution. The principle of UV-Vis spectrophotometry is based on the interaction of electromagnetic radiation with molecules containing chromophore groups. Light energy at specific wavelengths is absorbed by electrons in the molecules, causing electrons to transition from a lower energy level to a higher energy level (Amin et al., 2023).

UV-Vis spectrophotometry operates based on the interaction of ultraviolet (UV) and visible light with molecules in the sample. When light of a specific wavelength passes through a solution, certain molecules absorb energy from that light, reducing the intensity of the transmitted light. The difference between the incoming and outgoing light intensities is measured as absorbance, which is directly proportional to the concentration and optical path according to the Beer-Lambert law. This principle allows the spectrophotometer to very sensitively and quickly determine the amount or characteristics of compounds in solution at wavelengths of 190–780 nm, including the UV and visible, based on their absorption patterns. Furthermore, in vitro SPF testing on leaf fractions, such as guava leaf fractions, using the UV-Vis method is performed by measuring absorbance in the UV-B wavelength range (approximately 290–320 nm), as this region is associated with UV radiation that causes adverse effects on the skin. The SPF value is then calculated using a mathematical equation (e.g., the Mansur equation) to indicate how effectively the fraction absorbs and protects against UV radiation. This is related to the content of secondary metabolites such as flavonoids and antioxidants that can absorb UV light (Wardani et al., 2025).

As a step in evaluating the photoprotective ability of a material, the SPF value is calculated for the crystal guava fruit fraction. SPF is a universal indicator that explains the effectiveness of a product or substance that acts as a UV protector. The higher the SPF value of a product or active ingredient in a sunscreen, the more effective it is in protecting the skin from the harmful effects of UV rays. SPF is defined as the ratio between the amount of solar energy (in this case UV-B) required to cause minimal erythema on sunscreen-protected skin and unprotected skin. The SPF value is used to describe the effectiveness of protection against ultraviolet radiation. The SPF value is indicated by a number that indicates how long a person's skin can withstand sun exposure. The higher the SPF value, the longer the protection provided to the skin from sun exposure (Sunariya, 2016).

Fractionated SPF (Sun Protection Factor) testing involves weighing 0.1 grams of the fraction sample, placing it in a 100 ml volumetric flask, and adding solvents of varying polarity to the mark. The solution is then diluted in stages, with 5 ml, 10 ml, 15 ml, 20 ml, and 25 ml of the stock solution being placed in a 25 ml volumetric flask and adding solvents of varying polarity to the mark. This results in varying test solution concentrations of 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm. The above treatment was performed on each fraction of water, ethyl acetate, and n-hexane. Then, the absorbance of each fraction solution was measured at a wavelength range of 290-320 nm (Rahman et al., 2025).

The results of the average SPF value test above prove that the three fractions of crystal guava fruit (Psidium guajava L.) have proven potential as Sun Protection Factors. Based on the average SPF value measurements above, it can be stated that in the water fraction the lowest SPF value is at a concentration of 200 ppm, namely 3.249 which is included in the minimum category because it is in the range (2-4), for the ethyl acetate fraction at a concentration of 200 ppm, the value is 13.363 which is included in the maximum protection category because it is in the range (8-15), and in the n-Hexane fraction at a concentration of 200 ppm, the value is 2.069 which is included in the minimum category because it is in the range (2-4). At a concentration of 400 ppm, the water fraction showed a value of 6.735, which falls into the extra protection category because it falls within the range (6-8). The ethyl acetate fraction itself showed a value of 19.377, which falls into the ultra protection category because it falls within the range (≥15). The n-hexane fraction obtained a value of 2.196, which falls into the minimum category because it falls within the range (2-4).

At a concentration of 600 ppm, the water fraction showed a value of 9.549, which falls into the maximum protection category because it falls within the range (8-15). The ethyl acetate fraction itself showed a value of 19.755, which falls into the ultra protection category because it falls within the range (≥15). The n-hexane fraction obtained a value of 3.488, which falls into the minimum category because it falls within the range (2-4). At a concentration of 800 ppm, the water fraction showed a value of 13.189, which falls into the maximum protection category because it is in the range (8-15), the ethyl acetate fraction itself showed a value of 23.505, which falls into the ultra protection

category because it is in the value (≥15), while the n-Hexane fraction got a value of 5.694, which falls into the medium protection category because it is in the range (4-6). And for the highest concentration at 1000 ppm and getting the highest SPF value too, the water fraction was 16.820, which falls into the ultra protection category because it is in the value (≥15), the ethyl acetate fraction showed the highest value of 24.249, which falls into the ultra protection category because it is in the value (≥15), and the n-Hexane got a value of 7.497, which falls into the extra protection category because it is in the range (6-8).

Based on the results of the values obtained in Table 4.12, it shows that the highest ultra protection was obtained by the ethyl astate fraction, this is because the ethyl acetate fraction is semi-polar so it is able to attract flavonoid compounds needed in determining the SPF value itself and has the potential as a sunscreen activity that can protect the skin against sun exposure for 4-5 hours (Handayani and Susiloningrum., 2025). In the results of the water fraction which showed only one concentration that showed ultra protection, because the water fraction extracted polar compounds such as sugars, glycosides, saponins, whose UV absorption capacity was lower than flavonoids which were semi-polar. So this likely requires a high concentration to obtain UV absorption efficiency, in contrast to the ethyl acetate fraction (Handayani and Susiloningrum., 2025). Meanwhile, the results of n-Hexane only showed ultra protection at the highest concentration because it is non-polar so it cannot attract flavonoids that affect UV absorption efficiency well (Marliana et al., 2022). According to Marliana et al., (2022), a compound is said to act as a sunscreen if it has a minimum SPF value of 2.

The Sun Protection Factor (SPF) value is influenced by the difference in sunscreen concentration. Each sample's concentration was replicated three times to increase the accuracy of the absorbance values in the study and to reduce the risk of error. Concentration also plays a role in the absorbance value of a sample. The higher the concentration, the higher the absorbance value. This factor can increase or decrease the UV absorption of each sunscreen (Suryadi et al., 2021).

A maximum SPF value indicates protection from UV rays by blocking 93.3-95.9% of UV radiation. An ultra SPF value can block 96.0-97.4% of UV radiation (Suryadi et al., 2021). The obtained absorbance values align with predictions based on the Beer-Lambert law, where $A = \log(I_0/I)$. An absorbance value of 1.0 indicates that 90% of UV light at a given wavelength is absorbed by the test sample, resulting in only 10% of the light being transmitted. This value is ideal for quantitative SPF calculations because it falls within the linear range of UV-Vis spectrophotometer measurements (Chemguide, 2026).

Test results revealed several secondary metabolites present in crystal guava (Psidium guajava L.) fruit, including flavonoids, terpenoids, and saponins. The presence of flavonoids in crystal guava fruit can be used as active compounds that act as sunscreen agents.

Research by Wardatun et al. (2023) showed that the ethyl acetate fraction from Carica papaya leaves had the highest SPF value compared to the n-hexane and water fractions. Furthermore, research by Amsiyah et al. (2021) indicated that the ethyl acetate extract of guava leaves had an SPF of 15.17 at a concentration of 250 ppm, categorized as ultra protection. This high SPF value is related to the presence of flavonoids. Thus, the ethyl acetate fraction of crystal guava fruit has high potential as an active ingredient in sunscreen due to its ability to effectively absorb UV radiation.

Research by Wardatun et al. (2023) also showed that the n-hexane fraction from Carica papaya leaves has a lower SPF value than the ethyl acetate fraction. Research by Shadrina et al. (2025) also showed that the n-hexane fraction from Surian leaves has a lower SPF value than ethyl acetate. The n-hexane fraction has the lowest SPF value and only shows moderate to extra protection at concentrations of 150–250 ppm, while the water fraction shows moderate to ultra protection with a maximum SPF of 24.5165 at a concentration of 250 ppm. This indicates that the semi-polar to polar ethyl acetate and water fractions are capable of extracting phenolic and flavonoid compounds, two groups of compounds that have been shown to be effective at absorbing UV radiation.

CONCLUSION

Based on the results of the evaluation of the SPF values of the water, ethyl acetate, and n-hexane fractions, it can be concluded that:

1. The water, ethyl acetate, and n-hexane fractions of crystal guava (Psidium guajava L.) fruit show potential as Sun Protection Factors (SPFs) because they are in the range of 2-4 (minimum category).
2. The best results were obtained at a concentration of 1000 ppm, with the ethyl acetate fraction having the highest SPF value of 24.249, categorized as ultra protection.
3. The ethyl acetate fraction shows potential as an active sunscreen ingredient.

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