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ACTIVITY TEST OF RED BETEL LEAF ETHANOL EXTRACT (*Piper crocatum* Ruiz & Pav) AS AN ACTIVE INGREDIENT IN SUNSCREEN PREPARATIONS

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ABSTRACT

Skin that is continuously exposed to sunlight can cause a reduction in the structure and elasticity of the skin and other bad effects, so it is necessary to have a skin protective agent, one of which is using sunscreen preparations. In the cosmetics sector, making products from natural ingredients is more profitable. They have a good tolerance for the skin because they do not cause severe irritation to sensitive skin. This research aims to determine the activity of red betel leaf ethanol extract as a sunscreen by looking at the SPF (Sun Protection Factor) value. The determination of the SPF value of red betel leaf ethanol extract was carried out *in vitro* using UV-Vis spectrophotometers at a wavelength range of 290 – 320 nm. Phytochemical testing is carried out to determine the content of chemical compounds that play a role in sunscreen activity. The research results show that the SPF values obtained from concentrations of 100, 200, 300, 400, 500, and 600 ppm, respectively, are 8.486 (extra-protection), 10,849 (maximum protection), 13,709 (maximum protection), 17.1 (ultra protection), 22 (ultra protection) and 25 (ultra protection). Based on the results of phytochemical tests, the ethanol extract of red betel leaves positively contains phenolics, flavonoids, alkaloids, tannins, saponins, steroids, and/or terpenoids.

Keywords: Red betel leaves (*Piper crocatum* Ruiz &Pav), Sunscreen, SPF, *In-Vitro*, Ultra-protection

INTRODUCTION

Indonesia is a country with a tropical climate and a high intensity of sun exposure. High sun exposure can cause sunburn or redness if unprotected (Resi Andela, 2023). Naturally, the tissue in the epidermal layer of the skin is able to counteract the adverse effects caused by UV exposure, such as during sweating, in the process of melanin formation, and during the thickening process in the stratum corneum layer. If the skin is exposed to sunlight continuously and without protection, the sunlight can penetrate into the hypodermis layer of the skin, causing structural and elasticity reduction of the skin, and other adverse effects such as potentially causing skin cancer (Fitri Yani and Dirmansyah, 2021). To reduce the negative effects caused by ultraviolet rays, it is necessary to have a skin protection material. One of them is by using sunscreen preparations (Mokodompit, Edy, and Wiyono, 2013).

SPF, or Sun Protection Factor, is a value that can be used as an indicator of sunscreen effectiveness. The greater the ability of a sunscreen to protect the skin against the negative effects of UV exposure, the higher value of SPF (Avianka, Mardhiani, and Santoso, 2022). A synthetic compound or a compound from extracted natural ingredients can have sunscreen effectiveness. In the field of cosmetics, natural ingredients are more

favorable to be used as the main ingredient of a product because they have a good tolerance for sensitive skin and do not cause severe irritation (Ekowati and Hanifah, 2017).

The use of antioxidant compounds in sunscreens can provide photo-protective effects. The use of substances that have antioxidant activity prevents the occurrence of several diseases caused by UV exposure. Antioxidant compounds in sunscreen preparations that are capable of becoming free anti radicals include flavonoids, cinnamates, tannins, interquinones, and others. Secondary metabolite compounds such as flavonoids and polyphenols have good potential as sunscreen agents. This is because these secondary metabolite compounds have aromatic groups conjugated with carbonyl groups. These compounds can stabilize electron transfer because they can absorb or reduce the intensity of UV radiation (Henry *et al.*, 2020).

One of the natural ingredients that can be used as sunscreen is red betel leaf (*Piper crocatum* Ruiz & Pav). In Indonesia, red betel leaves are widely used as traditional medicine, germicides, anticancer drugs and to treat several infectious diseases. This potential comes from secondary metabolites, especially alkaloids, saponins, flavonoids, and essential oil content (Januarti *et al.*, 2019).

RESEARCH METHODS

Equipment and Materials

List the large or special equipment used in the research, including the brand, type, and specifications. Common laboratory equipment, such as glassware, droppers, scalpels, and the like, need not be mentioned. Specific/special equipment, such as custom-designed or modified standard equipment, should be accompanied by a diagram/photo of the equipment. The degree and specifications of the materials, brand, and supplier for each material must be mentioned. The gender, strain, age, average weight, and SD of the test animals should be reported in this section. If the crude drugs were obtained by self-collection from living plants, as far as possible, the age of the plant,fruit,leaf position, month of collection, and time of collection should be mentioned. If purchased, the source of the purchase should be stated.

The materials used in this study were red betel leaf simplicia, 96% ethanol (Bratachem), FeCl3 1% and 5% (Merck), dragendroff reagent (Merck), mayer reagent (Merck), HCl (Bratachem), 70% ethanol (Bratachem), 25% ammonia (Bratachem), chloroform (Bratachem), Mg powder (Merck), alcohol-HCl solution (1: 1), gelatin solution, Steasny reagent (Merck), 1N NaOH (Bratachem), 30% NaOH (Bratachem), anhydrous Na_2SO_4 (Bratachem), anhydrous CH_3COOH (Merck), H_2SO_4 (p) (Merck), chloroform ether (2:1) (Bratachem), benzene (Bratachem), distilled water.

Research Procedure

1. Collection and Determination of Test Plants

   The test plants were red betel leaves obtained from Kubang Hamlet, Sukajaya Village, and South Sumedang District. Red betel plants were determined at the Herbarium Jatinangor Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics, Padjadjaran University.
2. Preparation of Ethanol Extract of Red Betel Leaf (*Piper crocatum* Ruiz &Pav)

   Red betel leaves that have been collected are then washed to remove impurities, washed with running water, and cleaned. Next, they are chopped into small parts to facilitate the drying process. Then ground using a blender into powder. The powdered simplicia is stored in a tightly closed container at room temperature. Red betel leaf simplicia was macerated using 96% ethanol for 3x24 hours. The liquid extract of red betel leaf was obtained, then evaporated to obtain a thick extract of red betel leaf (Wijianto, Nurhidayah, and Luliana, 2022).
3. Phytochemical Screening of Simplicia and Extract of Red Betel Leaf (*Piper crocatum*

Ruiz & Pav)

Phytochemical screening procedures for simplicia and ethanol extracts of red betel leaves according to Wijianto et al (2022) and Simorangkir et al (2017) including alkaloids, flavonoids, saponins, tannins, steroids / triterpenoids (Simorangkir *et al.*, 2017) (Aji Najihudin *et al.*, 2023).

4. Sunscreen Activity Test on Red Betel Leaf Extract (*Piper crocatum* Ruiz &Pav)

Red betel leaf extract was diluted with ethanol p.a. as much as 100 mL. Dilutions were carried out to obtain concentrations of 600 ppm, 500 ppm, 400 ppm, 300 ppm, 200 ppm, and 100 ppm. The extract was measured using UV-Vis spectrophotometry to determine the SPF every 5 nm in the wavelength range of 290-320 nm, and the absorbance value was recorded (Rantika *et al.*, 2020).

Data Analysis

The SPF value was calculated using Manshur's equation (11).

$$SPF = CF \; X \sum_{320}^{290} (EE \; x \; I \; x \; A)$$

Description:

CF : Correction Factor

EE : Erythemal Effect Spectrum

I : Intensity Spectrum of the Sun

A : Absorbance of Sample

The value of EE X I is constant and is shown in **Table I** below:

Table I. Normalized Product Function Used in SPF Calculation

No	Wavelength (λ nm)	EE x I
1	290	0.0150
2	295	0.0817
3	300	0.2874
4	305	0.3278
5	310	0.1864
6	315	0.0839
7	320	0.0180
Total		**1**

Calculation method:

1. The absorption value obtained is multiplied by the EE x I value for each wavelength contained in the table above.
2. The result of multiplying the uptake by EE x I is summed up.
3. The sum result is then multiplied by a correction factor whose value is 10 to get the SPF value of the preparation.

Data analysis using one-way ANOVA was used to determine the average difference in the average SPF value of the cream obtained against the concentration of extract used.

RESULTS AND DISCUSSION

1. Preparation and Extraction of Red Betel Leaf (*Piper crocatum* Ruiz &Pav)

This study was conducted using red betel leaf simplicia obtained in Kubang Hamlet, Sukajaya Village, South Sumedang District, West Java. Before being used, the

simplicia was first determined at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, FMIPA, Padjadjaran University. The purpose of determining is to ensure the identity of simplicia or plants that will be used in research so that errors in collecting data on the tested material can be avoided. The results of plant determination show that the plant is a type of *Piper crocatum* Ruiz & Pav, which comes from the Piperaceae family.

Simplicia powder from red betel leaves is extracted by maceration for 3x24 hours, using a 96% ethanol solvent, replacing the solvent every 1x24 hours, and occasionally stirring, which aims to maximize the withdrawal process of active substances by the solvent. The ethanol used in this study acts as a solvent because it has semi-polar properties with a polarity index value of 5.2, so it can extract compounds with various polarity values. Ethanol solvents have a good enough ability to penetrate plant cell walls, so the process of separating secondary metabolites from natural materials is easier to do (Ayu Rismiasih, 2022).

The obtained macerate is then filtered using filter paper so that a dilute filtrate is obtained. The dilute filtrate is then evaporated to concentrate the extract and remove the solvent contained. The yield of the ethanol extract of red betel leaf is 12%.

Table II. Yield of Ethanol Extract of Red Betel Leaf (*Piper crocatum* Ruiz & Pav)

Weight of Simplicia (g)	Weight of Viscous Extract (g)	Yield (%)	Extract Color
802,72	98,2	12	Deep green

2. Phytochemical Test of Ethanol Extract of Red Betel Leaf (*Piper crocatum* Ruiz & Pav)

A phytochemical test is a test conducted to determine the content of chemical compounds contained in a sample. The results of the phytochemical test of the ethanol extract of red betel leaf can be seen in **Table II**.

The results of phytochemical screening of ethanol extracts of red betel leaf show that phenolic compounds, flavonoids, alkaloids, tannins, saponins, and steroids and triterpenoids can be attracted in ethanol solvents. This is because ethanol solvents have semipolar properties, so they can attract active components with various polarities and extract them more perfectly (Resi Andela, 2023).

Table III. Phytochemical Screening Results of Ethanol Extract of Red Betel Leaf (*Piper crocatum* Ruiz & Pav)

No.	Secondary Metabolites	Extract
1	Phenolic	Positive
2	Flavonoids	Positive
3	Alkaloids	Positive
4	Tannins	Positive
5	Saponins	Positive
6	Steroids/Triterpenoids	Positive
7	Quinone	Negative

3. Sunscreen Activity Test of Ethanol Extract of Red Betel Leaf (*Piper crocatum* Ruiz & Pav)

SPF is a quantitative measure of the effectiveness of a material that has activity as a sunscreen. The SPF value of red betel leaf ethanol extract is presented in **Table III**. The in-vitro SPF values of red betel leaf ethanol extract sunscreen cream preparations with various concentrations obtained were subjected to statistical analysis, namely one-way ANOVA. This test was conducted to determine if there was a significant difference in the in-vitro SPF value of each concentration. The normality test obtained a significance value> 0.05 which indicates that the data is normally distributed. The homogeneity test has a significance value >0.05 which indicates that the data variance is homogeneous.

Furthermore, the one-way ANOVA test obtained a significance value of 0.000, indicating a significant difference among the 6 concentrations of red betel leaf ethanol extract on the resulting SPF value.

Table IV. SPF Value of Ethanol Extract of Red Betel Leaf (*Piper crocatum* Ruiz & Pav)

Extract Concentration (ppm)	SPF Value	Protection Type (1)
100	8,486	Extra protection
200	10,849	Maximum protection
300	13,709	Maximum protection
400	17,100	Ultra protection
500	22,000	Ultra protection
600	25,000	Ultra protection

Based on the results presented in the table above, it can be seen that red betel leaf ethanol concentrate has the best sunscreen effect at a concentration of 600 ppm and is classified as ultra-protection (SPF value ≥ 15) of 25, followed by concentrations of 500 ppm and 400 ppm at the same protection with SPF values of 22 and 17.1, respectively. At concentrations of 300 ppm and 200 ppm classified as maximum protection with SPF values of 13.709 and 10.849, respectively, and at a concentration of 100 ppm classified as extra protection with an SPF value of 8.486.

According to More et al. (More *et al.*, 2013), one of the variables used to determine the SPF value is the concentration of sunscreen ingredients, This variable can increase or decrease UV absorption. In absorbance measurements in the wavelength range of 290-320 nm, the results of the red betel leaf ethanol extract sunscreen test showed that the concentration value and SPF value were directly proportional. The smaller the concentration, the lower the SPF value, and the greater the concentration, the higher the SPF value. This is because at high concentrations, the secondary metabolites in the extract have more chromophore groups, which can cause the absorbance value to be higher (Nasution *et al.*, 2020). Thus, this is in accordance with the results obtained, which can be seen in **Figure 1**.

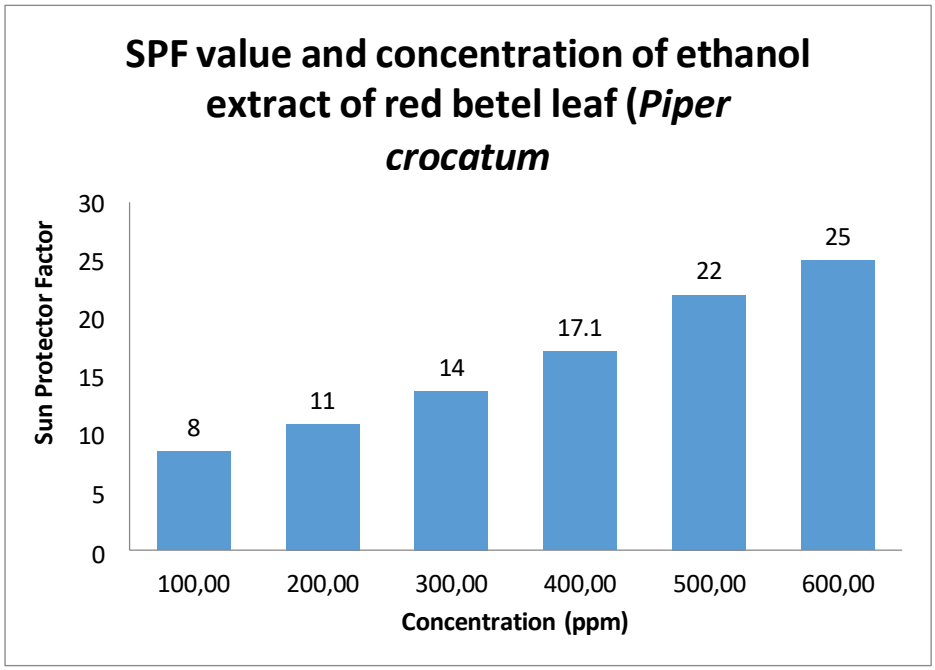

Figure 1. Diagram of the relationship between SPF value and concentration of ethanol extract of red betel leaf (*Piper crocatum* Ruiz & Pav).

The selection of sunscreen based on the SPF value multiplied by 10 minutes shows the length of sunscreen resistance in protecting the skin, where sunscreen is considered good if it has an SPF value above 15. The results of the sunscreen test obtained at a concentration of 100 ppm have an SPF value of 8.486, which means it can protect the skin for 84.86 minutes. Concentration of 200 ppm with SPF value 10.849; which means it can protect the skin for 108.49 minutes, concentration of 300 ppm with SPF value 13.709; which means it can maintain the skin for 137.09 minutes, concentration of 400 ppm with SPF value 17.1; which means it can maintain the skin for 171 minutes, and concentration of 500 ppm with SPF value 22, which can protect the skin for 220 minutes. In this study, the highest SPF value of red betel leaf ethanol extract is at a concentration of 600 ppm, with an ultra-protection category that can protect the skin for 3-4 hours at UV B wavelengths (Resi Andela, 2023).

The results of the analysis showed that the ethanol extract of red betel leaf has the potential to be used as an active ingredient in sunscreen preparations. This is due to the presence of a secondary metabolite compound contained in the ethanol extract of betel leaf. One of the metabolites, namely phenolic compounds, has photoprotective properties that can prevent adverse effects due to UV exposure (Injilia Wungkana, Edi Suryanto, 2013).

Flavonoids are phenolic compounds (C_6H_5OH) that form conjugated bonds in the benzene nucleus, where, when absorbing UV light, electron transfer will occur.

Similarities in conjugated systems in chemical compounds contained in sunscreen preparations can provide sunscreen effects (Injilia Wungkana, Edi Suryanto, 2013).

Tannins belong to a group of polyphenols that have potential as antioxidants with a strong category that plays a role in cell protection against damage by free radicals, so that the risk of skin cancer and premature aging can be lowered. The mechanism of action of tannin on the skin epidermis is by reducing the amount of H_2O_2, which inhibits the induction of ornithine decarboxylase and increases DNA synthesis (Injilia Wungkana, Edi Suryanto, 2013).

In addition, the content of other secondary metabolite compounds such as flavonoids, alkaloids, glycosides, saponins, tannins, and triterpenoids has a chromophore group or conjugated single double bond that can absorb ultraviolet light so as to reduce the adverse effects of UV radiation on the skin (Yuni and Yani, 2021).

CONCLUSION

The results showed that the SPF values obtained from concentrations of 100, 200, 300, 400, 500, and 600 ppm were 8.486 (extra protection), 10.849 (maximum protection), 13.709 (maximum protection), 17.1 (ultra-protection), 22 (ultra-protection), and 25 (ultra-protection), respectively. Ethanol extract of red betel leaf (*Piper crocatum* Ruiz &Pav) has potential as a sunscreen and can be used as an active ingredient in the preparation of sunscreen preparations.

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REFERENCES

Aji Najihudin *et al.* (2023) 'KARAKTERISASI DAN STUDI PENAPISAN FITOKIMIA DAUN KELOR (Moringa oleifera L.) ASAL GARUT JAWA BARAT', *Medical Sains : Jurnal Ilmiah Kefarmasian*, 8(2), pp. 679–686. Available at: https://doi.org/10.37874/ms.v8i2.761.

Avianka, V., Mardhiani, Y.D. and Santoso, R. (2022) 'Studi Pustaka Peningkatan Nilai SPF (Sun Protection Factor) pada Tabir Surya dengan Penambahan Bahan Alam', *Jurnal Sains dan Kesehatan*, 4(1), pp. 79–88. Available at: https://doi.org/10.25026/jsk.v4i1.664.

Ayu Rismiasih, A.K.J. (2022) 'Uji Aktivitas Tabir Surya Ekstrak Etanol Daun Matoa (Pometia pinnata) secara in vitro', *Jurnal Komunitas Farmasi Nasional*, 2(1), pp. 213–215.

Ekowati, D. and Hanifah, I.R. (2017) 'POTENSI TONGKOL JAGUNG (Zea Mays L.) SEBAGAI SUNSCREEN DALAM SEDIAAN HAND BODY LOTION', *Jurnal Ilmiah Manuntung*, 2(2), pp. 198–207. Available at: https://doi.org/10.51352/jim.v2i2.67.

Fitri Yani, D. and Dirmansyah, R. (2021) 'Uji Aktivitas Fraksi Metanol dan N-Heksan Kulit dan Kernel Biji Kebiul (Caesalpinia bonduc L.) sebagai Tabir Surya', *Jurnal Sains Dasar*, 10(1), pp. 1–5. Available at: https://doi.org/10.21831/jsd.v10i1.39065.

Henry, D. *et al.* (2020) 'POTENSI AKTIVITAS TABIR SURYA EKSTRAK DAUN DAN KULIT BATANG DENGEN (Dillenia serrata) SECARA IN VITRO', *Journal of the European Academy of Dermatology and Venereology*, 34(8), pp. 709.e1-709.e9. Available at: http://dx.doi.org/10.1016/j.jaad.2013.01.032.

Injilia Wungkana, Edi Suryanto, L. (2013) 'AKTIVITAS ANTIOKSIDAN DAN TABIR SURYA FRAKSI FENOLIK DARI LIMBAH TONGKOL JAGUNG (Zea mays L.)', *PHARMACON Jurnal Ilmiah Farmasi – UNSRAT Vol. 2 No. 04 November 2013 ISSN 2302 - 2493*, 2(04), pp. 149–155.

Januarti, I.B. *et al.* (2019) 'Potensi Ekstrak Terpurifikasi Daun Sirih Merah (*Piper crocatum* Ruiz &Pav) Sebagai Antioksidan Dan Antibakteri', *JPSCR : Journal of Pharmaceutical Science and Clinical Research*, 4(2), p. 60. Available at: https://doi.org/10.20961/jpscr.v4i2.27206.

Mokodompit, A.N., Edy, H.J. and Wiyono, W. (2013) 'Penentuan Nilai Sun Protective

Factor (SPF) Secara In Vitro Krim Tabir Surya Ekstrak Etanol Kulit Alpukat', *Jurnal Ilmiah Farmasi*, 2(3), pp. 83–85.

More, B.H. *et al.* (2013) 'Evaluation of Sunscreen activity of Cream containing Leaves Extract of Butea monosperma for Topical application', *International Journal of Research in Cosmetic Science*, 3(1), pp. 1–6. Available at: http://www.urpjournals.com.

Nasution, M.R. *et al.* (2020) 'Penentuan Aktivitas Tabir Surya Ekstrak Etanol Daun Marpuyan (Rhodamnia cinerea Jack.) secara In Vitro', *Jurnal Dunia Farmasi*, 4(2), pp. 59–67. Available at: https://doi.org/10.33085/jdf.v4i2.4599.

Rantika, N. *et al.* (2020) 'Formulasi dan Penentuan Nilai SPF Sediaan Lotion Ekstrak Sari Buah Jeruk Manis (Citrus x aurantium L.) Sebagai Tabir Surya', *Journal of Current Pharmaceutical Sciences*, 4(1), pp. 2598–2095.

Resi Andela, D.I.D. yani (2023) "Alchemy : journal of chemistry", 07.

Simorangkir, M. *et al.* (2017) '*Jurnal Pendidikan Kimia (JPKim) Analisis Fitokimia Metabolit Sekunder Ekstrak Daun dan Buah Solanum blumei Nees ex* Blume lokal Secondary Metabolites Phytochemical Analysis of Leaves and Fruit Extract Solanum blumei Nees ex Blume Local', 9(1), pp. 244–248. Available at: http://jurnal.unimed.ac.id/2012/index.php/.

Wijianto, B., Nurhidayah, A.L. and Luliana, S. (2022) 'Standardization of Secondary Metabolites and Heavy Metal Contamination Assay on Onchidiid Slug (Onchidium Typhae) West Kalimantan Waters', *Jurnal Farmasi Sains dan Praktis*, 8(3), pp. 199–206. Available at: https://doi.org/10.31603/pharmacy.v8i3.7296.

Yuni, R.T. and Yani, D.F. (2021) 'Uji Fitokimia dan Penentuan Nilai Sun Protection Factor (SPF) Fraksi Metanol dan n-heksan Daun Kebiul (Caesalpinia Bonduc) Secara In Vitro', *Fullerene Journal Of Chemistry*, 6(2), pp. 71–75. Available at: https://doi.org/10.37033/fjc.v6i2.251.