

# Anti UV, the Sun Protection Factor (SPF), and Irritation Test of Coastal Vegetation *Ricinus communis*

Easter C. H. Situmorang<sup>1</sup>, Robert A. Bara<sup>1\*</sup>, Veibe Warouw<sup>1</sup>, Billy T. Wagey<sup>1</sup>, Desy M.H. Mantiri<sup>1</sup>, Calvyn F.A. Sondak<sup>1</sup>, Christi D. Mambo<sup>2</sup>, and Jimmy Posangi<sup>2</sup>

<sup>1</sup>Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Bahu, 95115 Manado, Indonesia

<sup>2</sup>Faculty of Medicine, Sam Ratulangi University, Jl. Kampus Bahu, 95115 Manado, Indonesia

**Abstract.** This study aims to test anti-UV activity, determine the SPF, and perform an irritation test on human skin from *Ricinus communis* leaf extract. The anti-UV and SPF values were determined using a UV-Vis Spectrophotometer. Furthermore, the irritation test was performed by observing the skin of human objects after cream with *R. communis* extract was applied. The results show that *R. communis* leaf extract has anti-UV-B activity, With SPF value categorized as high according to the US FDA at a concentration of 1000 ppm. Furthermore, observations from 20 participants from the patch test found no skin reactions such as edema, urticaria, irritation, and erythema on any of the objects. The *Ricinus communis* extract has the potential to develop as a natural sunscreen for human use.

## 1. Introduction

Vegetation on the coast is a group of plants that inhabit tidal areas to land areas or islands still influenced by the sea. Generally, coastal vegetation is divided into three: true mangroves, associated mangroves, and non-mangrove vegetation. Non-mangrove coastal vegetation is a group of plants that live on sandy beaches, consisting of grass, *Terminalia catappa*, sea cypress, and coconut [1]. Associated mangroves are non-wood or climbing plants that live in coastal environments and mangrove forests. The associated mangrove group does not meet the characteristics of true mangroves. Associated mangroves are glycophytes with a particular salt tolerance, meaning that these plants are easily damaged by high salinity [2]. *Ricinus communis* is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India. It is commonly found in disturbed areas, such as dunes, road margins, shrublands, and coastal areas. *R. communis* is included in the associated mangroves that thrive in tropical climates [3]. A fast-growing shrub can reach the size of a small tree of about 12 m, with glossy leaves, 15-45 cm long, long petiole. The leaf is palmately lobed with 5-12 lobes and toothed margins. Young foliage is reddish purple and gradually changes to dark green as they

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\* Corresponding author: [robert.bara@unsrat.ac.id](mailto:robert.bara@unsrat.ac.id)

mature. The leaves are traditionally used to treat abdominal discomfort and constipation. It is also used to treat fevers, bronchitis, coughs, and skin diseases and has been proven to contain flavonoids, alkaloids, terpenoids, and phenolic compounds [4].

UV rays are part of the sunlight spectrum that harms human skin. Excessive exposure to UV rays causes skin problems such as hyperpigmentation and redness and, in the long term, can pose a risk of skin cancer [5]. Sunscreen can absorb approximately 85% of UV-B rays. The effectiveness of sunscreen is determined by the Sun Protection Factor (SPF) value, which shows the sunscreen's ability to protect the skin from erythema, significantly protecting against UV-B rays and not specifically protecting against UV-A rays [6]. The criteria for a sunscreen that consumers seek include being easy to apply, softening and moisturizing the skin, not causing allergies, and being easily absorbed into the skin. Sunscreen from natural ingredients and high SPF will match the market's needs [7]. WHO recommends using sunscreen as an early preventive measure to protect the skin from UV exposure [8]. Sunscreens work by absorbing and reflecting UV rays. The ability of sunscreen to protect the skin from UV rays is measured by the SPF or Sun Protecting Factor value [9]. This research was conducted based on this background.

*R. communis* is a plant that can be found elsewhere including coastal areas. *R. communis* is included in the mangrove-association group that thrives in tropical climates [3]. This plant's leaves contain flavonoids, alkaloids, terpenoids, and phenolic compounds [4]. *R. communis* can reduce the effects of sunburn and also stimulate collagen production, which can help soften and moisturize the skin [10]. Based on this description, *R. communis* leaves can be used for sunscreen.

*R. communis* is an herbal plant in the form of a small tree with a height of about 2 meters; after one year, it reaches 3-4 meters. The leaf bones are fingered with pointed and serrated edges. The diameter of the leaves is 15-30 cm with a 30 cm long stalk. The leaves and stems are reddish-green. Flowers are found in the upper leaf axils of 15-30 cm length. The fruit is a round capsule that is first green, then turns brown, and is covered with soft spines measuring 12-18 mm [3]. This shrub can grow at seashore to about 2,500 meters above sea level. It can also be found on the margins of mangrove forests. This species is also a mangrove associate. The leaves of *R. communis* can be used to treat headaches, as a poultice for ulcers, as rheumatic drugs, as animal feed, and as silkworm feed [3]. *R. communis* also has antioxidant activity that can neutralize free radicals [11].

A new product must undergo safety testing and risk assessment, before introduction, including skin irritation or allergy to the ingredients contained in the product. In 1944, Draize published a method of testing rabbit skin irritation and corrosion *in vivo*. The Draize test is already considered a reference for determining the irritation potential of chemicals in humans, but this test has some disadvantages. Rabbit and human skin have different natural abilities to allow liquids to pass through, which may account for most of the increased irritation in the rabbit test. Rabbit skin is more permeable than human skin [12]. In 1998, an irritation test method was developed using human subjects, namely the human 4-hour patch test. This test was designed to avoid a greater mild irritation response. The advantage of this 4-hour patch test approach is that the results obtained in determining skin irritation are considered the most accurate compared to animal tests [13]. Human skin irritation tests have also been carried out by attaching the test material as mangosteen fruit peel extract to the arm skin of 6 volunteers consisting of men and women for 4 hours [1].

This study aimed to determine anti-UV activity, SPF value, and assessment of human skin irritation or allergy to sunscreen cream from *R. communis* leaves.

## 2. Methods

### 2.1 Location and time of research

Samples of *R. communis* leaves were taken from Sario Tumpaan Beach, Sario District, Manado City, with coordinates N 1°28'5.07"; E 124°49'47.85" (Figure 1). The tree was approximately 5 m tall. Samples were identified based on morphological characteristics through the Mangrove guidebook for Southeast Asia[8]. The extraction procedure was performed at the Marine Biotechnology and Pharmaceutics Laboratory, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Indonesia. The extract's anti-UV activity and SPF value test were conducted at the Integrated Laboratory Unit, Sam Ratulangi University. The HPLC analysis was performed at Forensic Laboratory Indonesian Police North Sulawesi Region This study was conducted for five months, from March to August 2023.

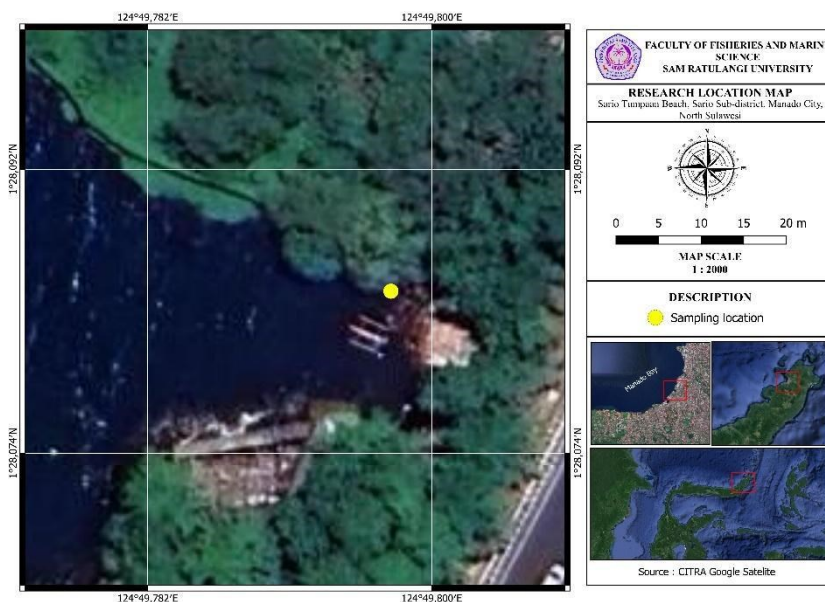


Fig. 1. Sampling location.

### 2.2 Sample collection and preparation

Leaves of *R. communis* were collected and washed thoroughly to remove dirt, cut into small pieces, and drained. The leaves were then dried using an oven at 40°C for 24 hours. The leaves were blended and weighed [14, 15].

### 2.3 Sample extraction

The leaf powder was dynamically extracted with ethyl acetate for 3 × 24 hours, protected from light, and shaken once a day[14, 15]. The leaf extract was filtered with filter paper to separate the filtrate from debris. The filtrate was evaporated using an oven at 40°C until the ethyl acetate evaporated entirely and the extract was obtained. The extract was weighed to determine its weight. The extract was freeze-dried at -45°C for 48 hours until dried [16].

## 2.4 Anti-UV activity test

The anti-UV test was carried out in vitro using the UV-Vis Spectrophotometer. The dried extract was dissolved with 95% ethanol at a concentration of 100 ppm. The maxima were analyzed at 280-325 nm for UV-A and 315-400 nm for UV-B.

## 2.5 Determination of SPF value

SPF value test was carried out by dissolving the extract in 95% ethanol at concentrations of 500, 1000, and 2000 ppm, respectively. The absorbance of each extract concentration was read using a UV-Vis spectrophotometer at a range of 290-320 nm with an interval of 5 nm in triple. After obtaining the average results of UV absorption in the extract, the SPF value was determined using the formula of [17, 18] as follows.

$$SPF = \frac{CF}{CF} \times \sum_{290}^{320} EE \times I \times abs \tag{1}$$

Description:

CF : Correction Factor (=10)

EE : Erythema Effect Spectrum

I : Light Intensity Spectrum

Abs : Absorbance of sunscreen sample (milli absorbance units/mAU)

**Table 1.** Levels of sunscreen ability based on SPF value determination. [19]

SPF	Product Category Designation
2 - < 12	Low
12 - < 30	Medium
≥ 30	High

Notes:

According to research by skin experts, SPF 1 in sunscreen cream can protect the skin for 5-10 minutes before being burned poon (Table 1).

2 - < 12 : low sun protection

12 - < 30 : medium sun protection

≥30 : high sun protection

## 2.6 Irritation test

The irritation test aims to determine the feasibility of *R. communis* leaf extract as a sunscreen product. Ethical approval was obtained from the Health Research Ethical Committee Prof. Dr. R. D. Kandou General Hospital Manado (approval number 272/EC/KEPK/VII/2023). The results of the irritation test in this study were supervised and consulted by a dermatologist and pharmacologist. Irritation test was carried out using the patch test method on twenty volunteers who met the following criteria [20], who had previously been anamnesis by a GP, and who had filled out an informed consent:

1. Males and females aged between 20-22 years. The twenty volunteers consisted of an equal number of both sexes.
2. Physically and mentally healthy.
3. Do not have a history of allergies.

**Table 2.** Formulation of cream preparations of *R. communis* leaf extracts.

Ingredient	Concentration (%)		
	F1	F2	F3
<i>R. communis</i> leaf extract	1	0,08	-
Cream base	99	99,91	100

*R. communis* leaf extract cream preparations can be seen in Table 2. The formulations were applied to the skin of the human arm for 6 hours. The reaction of the cream to the skin, such as irritation, edema, and erythema, was observed during and after the test. Symptoms noted were rash or itching, swollen and raised skin areas, small red spots, itching, stinging, or burning sensation, and dry or cracked skin. Furthermore, an assessment was made based on the International Contact Dermatitis Research Group (ICDRG) grading system [21].

The cream base used in this study contains the following ingredients: Water, Sorbitol, vegetable emulsifiers, e.g., mono and diglycerides fatty acids, polysorbate 80, and propylene glycol [22].

### 2.7 HPLC analysis

HPLC was carried out using the Waters<sup>tm</sup> e2695 separation module with a single-phase UV detector. XSelect CSH C18 Column was used with a 0.25 ml/min solvent flow rate. The dried extract was prepared by diluting it with HPLC-grade methanol prior to injection. The solvent elution used in this method was isocratic with 9:1 MeOH: water for 20 minutes of running time.

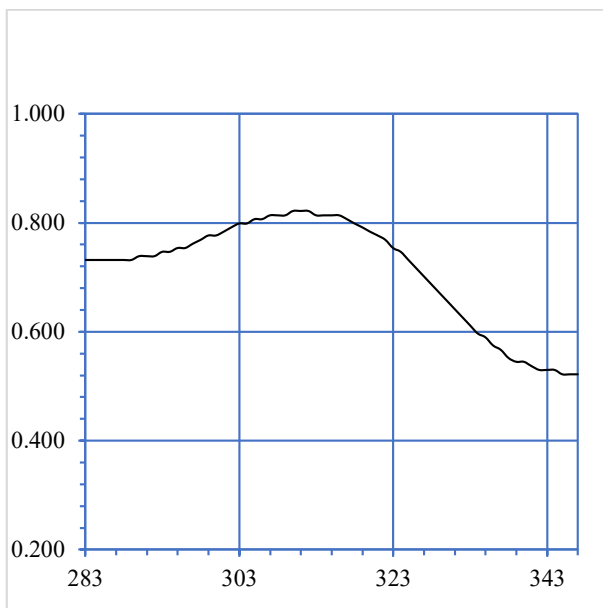
## 3. Results and discussions

The leaves of *R. communis* are dark green, including fingered leaf bones and serrated leaf margins. The characteristics of *R. communis* fruit are green with soft spines, while dried fruit is brown. The stem of the plant is reddish green (Figure 2).



**Fig. 2.** *R. communis*. A = leaf, B= fruit of *R. communis*.

Anti-UV activity testing: *R. communis* extract showed an absorption maximum at a wavelength of 307 nm with a value of 0.85 mAU (Figure 3), categorized as anti-UV-B active. Plants exposed to solar UV radiation produce anti-UV compounds as a form of plant



**Fig. 3.** The graph of the UV absorption maximum of *R. communis* leaf extract.

adaptation. These anti-UV compounds can be used as ingredients in sunscreen preparations that function to reduce the adverse effects of solar UV radiation. Based on the UV absorption, the related plant has anti-UV activity. Based on that result, the SPF value of the extract is determined. Table 3 shows the SPF value of *R. communis* leaf extract.

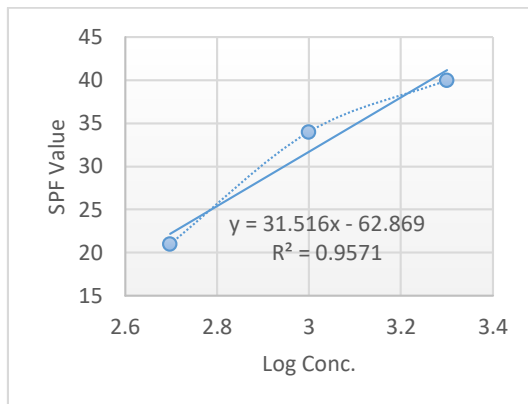
**Table 3.** SPF values of *R. communis* leaf extracts.

Sample	Concentration (ppm)	SPF value	UV protection Category [7]
<i>R. communis</i> leaf extract	500	21	Medium
	1000	34	High
	2000	40	High

This study shows the results of absorbance values with SPF values that increase linearly to log ppm concentration. Based on these data, the SPF value is categorized according to [18]. The results obtained by calculating Mansur’s formulation [18] on the extract of *R. communis* leaves at a concentration of 500 are included in moderate protection with an SPF value of 21 and SPF at concentrations of 1000 and 2000 ppm of 34 and 40, respectively, are included high protection.

In tropical and subtropical countries, it is recommended to use sunscreen with SPF 30, which aims to provide maximum protection against erythema and skin cancer [19]. An SPF value of 1 is considered safe for skin exposure to sunlight, such as skin damage and burning due to sun exposure within 5 minutes [23]. Using sunscreen cream from *R. communis* extract with a concentration of 1000 ppm or SPF value of 34 may allow a person to use it for 34 times 5 minutes, which is about 170 minutes without the effects of burning due to sun exposure. After that time, reapplication of sunscreen cream is needed. The concentration of *R. communis* leaf sample solution required for SPF 30 value is 884 ppm based on the following graph (Figure 4).





**Fig. 4.** Determination of SPF 30 value of *R. communis* leaf extract.

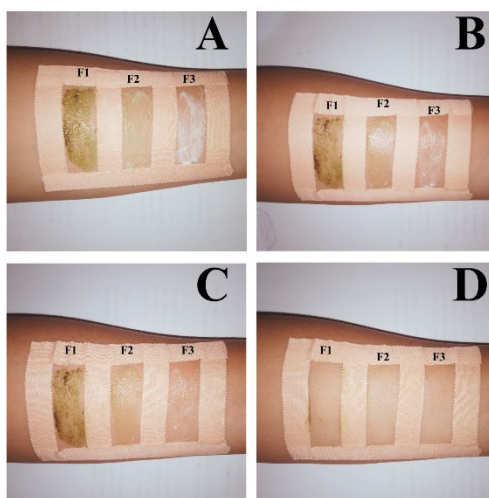
$$Y = 31.516x - 62.869$$

$$30 = 31.516x - 62.869$$

$$X = 2.946$$

$$SPF30 = 884 \text{ ppm} \cong 0.088\%$$

We performed an SPF = 30 For the human test. It was made at a concentration of extract:cream base = 0.088%. Furthermore, selecting a 1% extract concentration was conducted to test whether the high concentration of 1% on cream base negatively affected human dermatology. The SPF value indicates the sunscreen's level of protection against sunburn. The SPF value on sunscreens only indicates UV-B protection, as the SPF value is determined through a protection test against sunburn caused by UV-B radiation. Sunscreens cream that passes the broad-spectrum test indicate UV-A protection is also present. Sunscreens labeled with "Broad Spectrum SPF" specify that they have protection from both UV-A and UV-B radiations [19]. The effects of skin irritation were observed after the cream was applied during 6 hours of testing. The result is presented in Figure 5.

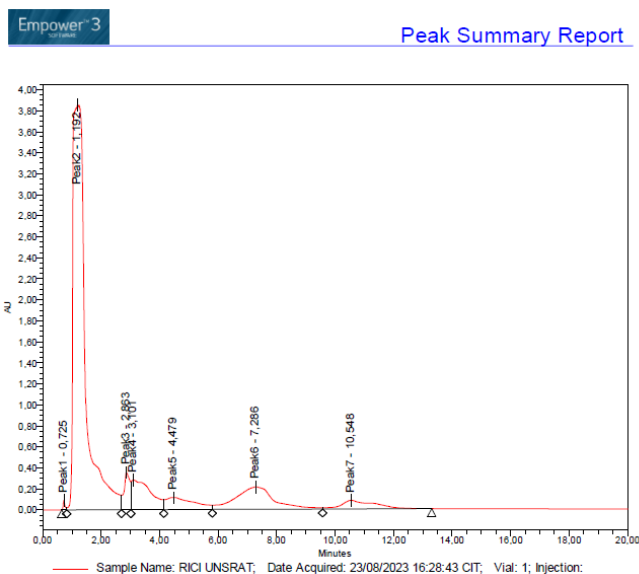


**Fig. 5.** Irritation test of 1% (F1) and 0.088% (F2) leaf extract cream and cream without added *R. communis* leaf extract (F3). A = Condition of the test arm at 0 hours; B = Condition of the test arm at 3 hours; C = Condition of the test arm at 6 hours; D = Condition of the arm after cleaning.

Irritation testing of *R. communis* leaf extract cream preparations with a concentration of 0.088% and 1% on twenty objects showed no skin abnormalities in all test participants. The testing of *R. communis* leaf extract cream at 0.088%, equal to an SPF value of 30, and at a high concentration of 1% on the participants' skin showed no edema, urticaria, irritation, or erythema was observed.

The observation results were followed base on the International Contact Dermatitis Research Group (ICDRG) grading system. From twenty participants, we did not find reactions such as irritation, edema, and erythema on the skin. The results of the irritation test were then consulted through direct discussion with a dermatologist and pharmacologist. *R.communis* samples are considered safe to be used as sunscreen cream ingredients. The particular plant is used by people in Tomini Village, Central Sulawesi, to reduce fever by patching the leaves on the skin [24].

Moreover, the dermatologist stated that irritation testing of sunscreen preparations on twenty objects did not fall into the category of skin disorders such as edema, urticaria, irritation, and erythema. The main characteristics of allergies are erythema and itching. Allergic skin disorders can be seen by inflammation that extends slowly, and the boundaries of inflammation are unclear [25].



**Fig. 6.** HPLC chromatogram of *R. communis* extract.

The HPLC chromatogram of *R. communis* ethyl acetate crude extract is shown in Figure. 6. Detected at 307 nm as of the related extract has seven compounds with only one primary compound. In the analysis, seven peaks were detected, and the peak with a retention time of 1.192 was identified as Rutin. Babu *et al.* (2017) stated the compound is predominantly present in the extract of *R. communis* [26]. Rutin-rich in *R. communis* plant showed DPPH and ABTS radical scavenging activities and efficiently reduced the DEX-induced myotube atrophy and mitochondrial oxidative damage in C2C12 cells in mice and prevent oxidative stress via Nrf2 signaling [27] inhibit cancer cell by regulating several cellular signaling pathway such as Wnt/ $\beta$ -catenin, p53-independent pathway, PI3K/Akt, JAK/STAT, MAPK, p53, apoptosis as well as NF- $\kappa$ B signaling pathways helps to mediate the anticancer impacts of this compound [28]. It also and shows the antiobesity effect to mammals [29]. Rutin is flavonoid glycoside, with a low molecular weight polyphenolic compound. This compound



is one of the natural antioxidants in the known natural class which is widely distributed in vegetables, fruits, and medicinal herbs [30]. Rutin may suspected to be responsible for the anti-UV activity of the *R. communis* plant.

## 4. Conclusion

Anti-UV activity testing on *R. communis* leaf extracts showed protection from UV-B rays. The extract has a high SPF value, providing extra protection. The *R. communis* extract cream irritation test found no skin abnormalities even at 1% of use.

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