Indonesian Journal of Cosmetic
E-ISSN: 3026-3352

FRAP REDUCING ACTIVITY OF *Protium javanicum* Burm.fil LEAVES: A SCREENING ANTIOXIDANT POTENCY OF NATURAL COSMETIC INGREDIENT

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ABSTRACT

Tenggulun (*Protium javanicum* Burm.fil) has been traditionally used to treat various ailments such as cough, stomachache, diarrhea, inflammation and headache. The leaves have therapeutic roles in the treatment and prevention of illnesses acting as antibacterial, antidiabetic and antiinflammation agents. It is also reported that tenggulun leaves can be used for insecticides, cosmetics and food additives. Recently, a global trend reported that natural products with antioxidant properties utilized as ingredients as whitening, antiaging, and antihyperpigmentation agent has been increasing. To explore utilization as cosmetic ingredients, this research aimed to determine antioxidant activity by reducing FRAP of the tenggulun leaves methanol extract using Trolox as the standard solution. The result showed that the tenggulun leaves extract contained secondary metabolites of flavonoid and polyphenolic. It confirmed by colorimetric reaction through magnesium and hydrochloric acid for flavonoids, and through FeCl3 1% for polyphenolics compound. The extract showed the FRAP reducing activity at 0,008634±0.00043 µmol TE/g DW. These results indicated the ability of tenggulun methanolic extract to reduce Fe (III) into Fe (II) equivalent to Trolox by the presence of secondary metabolites confirmed as flavonoid and polyphenolics. By this activity, *P. javanicum* showed potential to be developed as both an active pharmaceutical ingredient and excipient in cosmetics. These potentials may be employed as protector ingredients against oxidative skin-damage and as natural preservatives in cosmetic formulations.

Key words: FRAP, Antioxidant, *P.javanicum* leaves, Oxidative-skin damage

INTRODUCTION

Medicinal plants are described as plants with organs that can be used for medicine with healing properties. The organs used for medicine may vary from the body, the leaves, the roots and the flowers (Lestari et al., 2021). Tenggulun (*Protium javanicum* Burm.fil) are among the plants with medicinal properties found in Indonesia. *P. javanicum* are commonly used in Indonesia as timbers for construction and construction tools, while its leaves are rarely used and most likely disposed of. Based on previous studies, the leaves are known to possess antiinflammation, antibacterial and antioxidant activity, but its antioxidant activity measurements are not yet researched. There are numerous studies done to determine the potential uses of tenggulun leaves based on its content. The oleoresins and gum resins in tenggulun leaves can be used for its antiinflammation properties, as tonic, and to relieve rheumatism and headache, even can be used as insecticides, cosmetics, food additives and antioxidant (Nurfitria et al., 2024; Yolanda Simamora et al., 2021).

Yolanda Simamora et al. (2021) conducted research using various solvents to determine its effects on antioxidant activities in tenggulun leaves (*Protium javanicum* Burm.fil) extract using maceration extraction method. The study found that the difference in solvents affected the antioxidant activities in tenggulun leaves extract using the DPPH method as a measurement for antioxidant activities. The same study reported by Yusasrini et al. (2025), the maximum antioxidant activity of *P. javanicum* showed by extracting the leaves by high concentration of polar solvent. That solvent contributes to yield high total phenolic and total flavonoid content responsibles for antioxidant.

The exploration of antioxidant activity of natural products has shown significant endeavor this past several decades. This might correspond to the curiosity and revolutionary viewpoint of natural product utilization in the cosmetic industry (McMullen & Dell'Acqua, 2023). Recently, a global trend reported that natural products with antioxidant properties utilized as cosmetic ingredients has been increasing. This activity found related to whitening effect, anti-aging properties, photoprotective against UV radiation, and anti-hyperpigmentation which were beneficial in cosmetic products (Mapoung et al., 2021).

Various methods have been developed to measure the total characteristics of antioxidants, but none are truly ideal. The measurements are used to detect different characteristics of antioxidants present in the sample; this explains the reason different methods of measurements may explore different mechanisms of antioxidants. With the FRAP method, the antioxidant contents of a sample can be detected based on its ability to reduce

Fe (III) ion into Fe (II) ion. Therefore, the strength of antioxidants of a certain compound can be illustrated by its ability in reduction (Maryam et al., 2015).

In recent times, there has been no research done to measure antioxidant activities of the methanolic extract of tenggulun leaves using the FRAP method. Thus, a study on determining the antioxidant activities of tenggulun leaves using methanol solution and the FRAP method was needed as a preliminary research on conduct natural product-based cosmetics ingredients.

MATERIAL AND METHODS

Material

P. javanicum leaves were collected from Wanasaba East Lombok, West Nusa Tenggara. The leaves used in this study were 3 to 5 stalks from the shoot and has light green color.

Methods

Simplicia Preparation

The sample was cleaned thoroughly by tap water and dried, cut into smaller pieces, and then sun dried after being covered with a black cloth. After that, the dry sample sorted, ground with a blender, then packed in an inert container.

Extraction Process

The methanolic extract of tenggulun leaves was made through maceration. 300 grams of tenggulun leaves simplicia powder was put in a closed container to be soaked in 3 liters of methanol (volume ratio of 1:10) for 48 hours. Throughout the maceration process, the container was agitated every 4 hours for 5 minutes. The extraction process was done with the container closed, in a room temperature and away from direct exposure to sunlight. After 48 hours, filtration was done using filter paper. The obtained extract was concentrated using a rotary evaporator in 40^{o}C at 100 rpm. The concentrated extract of tenggulun leaves was then weighed and calculated for the yield.

Phytochemical Screening

Each extracted sample weighing 0,5 grams was transferred into 2 test tubes and then diluted with 10 mL ethanol. A total of 0,5 mL diluted sample was pipetted and then added with 1 mL ethanol, 0,5 grams magnesium powder and 1 mL of concentrated HCl. A positive result of flavonoid was confirmed if a yellow, red, or orange discoloration was identified. Phenolic identification was conducted using a total of 0,5 mL diluted sample added with 1mL ethanol

and 4 drops of $FeCl_3$ 1%. A positive result was confirmed if a black or dark blue discoloration was identified (Putri et al., 2023).

Preparation of FRAP Reagents
Buffer Acetate 300 mM pH 3,6
455,30 mg of natrium acetate anhydrous was dissolved with 50 mL of distilled water, and then added with 3,97 mL glacial acetic acid. It was then diluted to 250 mL to create an acetic buffer solution with a concentration of 0,30 mol/mL (Xiao et al., 2020).

Preparation of TPTZ solution
TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) was weighted to obtain 156,20 mg and was dissolved with distilled water and added with 0,17 mL concentrated HCl. The solution was diluted into 100 mL to create a TPTZ solution with a concentration of 10 mmol/L (Xiao et al., 2020).

Preparation of $FeCl_3$ Solution
$FeCl_3 6H_2O$ weighing 270,03 mg was dissolved with H_2O and diluted into 50 mL. The concentration of $FeCl_3$ was 20 mmol/mL (Xiao et al., 2020).

FRAP Reagent
The FRAP reagent was made by combining the acetic buffer solution, TPTZ solution, and the $FeCl_3$ with a volume ratio of 10:1:1. 25 mL of acetic buffer solution 0,3 M pH 3, 2,5 mL TPTZ solution (10 mM and 40 mM HCL), and 2,5 mL $FeCl_3 6H_2O$ 20 mM (Xiao et al., 2020).

Preparation of Trolox Standard Solution
600 µM of stock solution was made by dissolving 15,02 mg of Trolox in ethanol, and then diluted to 10 mL in the volumetric flask (Syarif et al., 2015).

FRAP Testing

The FRAP reagents were reacted with 3 mL FRAP with 1 mL sample and then incubated at 37^0C for 30 minutes. The Trolox standard solution was treated similarly with the sample (Xiao et al., 2020). And then measure the absorbance on the wavelength at the maximum value of 596 nm with the UV-Visible spectrophotometer (Yolanda Simamora et al., 2021). The blank solution that was used was combined with water that was incubated at 37^0C for 30 minutes. The Trolox standard solution was made to generate a standard curve, which would be used to determine the antioxidant capacity equivalent to Trolox.

Data Analysis

Antioxidant activities were measured with FRAP values calculation using following equation:

$$Frap\ value\ (\mu mol\ TE/g\ DW) = \frac{c\ x\ V\ x\ t}{m}$$

Where *c* was the Trolox concentration (µmol/ml) from the standard curve of the diluted samples, *V* was the sample volume (mL), *t* was the dilution factor, and *m* was the weight of the dry sample (g)(Xiao et al., 2020).

RESULT AND DISCUSSION

Tenggulun Leaves Simplicia and Extraction

The final weight of the tenggulun leaves simplicia was 620 grams out of 2 kg of wet simplicia. The result of the tenggulun leaves extraction was 23,755 grams and 7,918% worth of yield (Table 1). Out of the 620 grams of simplicia obtained, 300 grams was used during the study. The amount was justified by Yolanda Simamora et al. (2021) who demonstrated the use of 300 grams of simplicia could generate an extract with high antioxidant activities.

Table 1. Yield value of tenggulun leaves extract

Tenggulun leaves powder (gram)	Concentrated extract (gram)	Yield (%)	Concentrated extract color
300	23,755	7,918	Dark green

Phytochemical Screening

The phytochemical screening results showed that the methanolic extract of tenggulun leaves, indeed, contained flavonoi and polyphenolic compounds. The results of the phytochemistry screening are shown in Table 2.

Table 2. Tenggulun leaves methanol extract phytochemistry screening results

Testing type	Reagent	Changes	Result
Flavonoid	Mg powder + concentrated HCl	Reddish orange was shown	+
Polyphenol	$FeCl_3$ 1%	Darkish brown or darkish green or darkish blue was shown	+

Based on the Table 2, the phytochemical screening results of secondary metabolite compounds in the methanolic extract of tenggulun leaves proved to contain flavonoid and polyphenols. The methanolic extract of tenggulun leaves showed positives results of Flavonoids based on the change in color to reddish orange or orange due to the addition of concentrated HCl and Magnesium powder which could turn into red or orange-colored flavilium salt (Andasari et al., 2020). The addition of concentrated HCl was done to hydrolyze O-glycosyl. Glycosylation would be halted by H^+ ion due to its electrophilic nature. The magnesium powder created a red, yellow, or orange-colored complex. The qualitative flavonoid testing used was based on the Wilstatter Cyanidin method using magnesium metal powder in a concentrated HCl acidic medium. The sample would be deemed containing flavonoid if it resulted in a reddish color being shown (Lindawati & Hudzaifah Ma'ruf, 2020). This study identified a reddish orange discoloration which indicated the presence of flavonoid. The presence of magnesium powder and HCl in the testing was used to reduce flavonoid, especially the benzopyrene core in the flavonoid structure (Dwitiyanti et al., 2019).

The polyphenol testing on the extract resulted positive based on the darkish discoloration shown in the process. The darkish discoloration was caused by the aromatic –OH group in the polyphenol compound that would react with $FeCl_3$ 1% solution and generate a blue or dark-colored complex which could be assumed to be hexaphenolic Fe(III) (Haryati et al., 2015; Nurhaliza et al., 2023).

Antioxidant Activity Measurement

The FRAP method is one of the ways to measure antioxidant activities in the sample effectively and efficiently with minimum cost. The antioxidant activity is measured using Fe(III) based reagents and the principle of reduction-oxidation reactions where Fe(III) is reduced into Fe(II) (Naji et al., 2020).

The antioxidant activity was measured by reacting the FRAP reagents with the tenggulun leaves samples. The FRAP reagents were made from the combination of TPTZ, $FeCl_3$, and buffer acetate. The addition of $FeCl_3$ acted as the source of Fe(III) ions that would be reduced by the antioxidants. Meanwhile, the addition of buffer acetate was due to its nature of maintaining the effective pH range of 3,6 to 5,6 for stabilizing Fe(III) solubility (Muflihunna et al., 2014). As it is known that to reduce Fe(III)-TPTZ into Fe(II)-TPTZ requires an acidic medium or low pH, so the usage of buffer acetate with the lowest effective pH of 3,6 was needed.

Among all the methods used to measure the ability to reduce Fe (III) into Fe(II), such as FRAP, the colorimetry method uses the ability of

antioxidant to reduce colorless complex ($Fe3^{+}$-(2,4,6-Tris(2-pirydyl)-s-triazine)$_2$) $^{3+}$ into dark blue colored complex ($Fe2^{+}$-$(TPTZ)_2)^{2+}$ in an acidic medium (Wojtunik-Kulesza et al., 2020). The discoloration was measured using a UV-Visible spectrophotometer at 596 nm. The result was measured based on the usage of ferrous ion standard solution and certain standard antioxidant (in most cases, Trolox). The results were stated in the FRAP unit of measurement, which defined 1 M ferric ion as 1 ferrous ion (Wojtunik-Kulesza et al., 2020).

Based on Table 3, it was concluded that the regression equation from the correlation graph between concentration (x-axis) and absorbance (y-axis). The regression value from the graph was used to determine the antioxidant activities.

Table 3. The absorbance results of trolox standard solution

Concentration (µM)	Absorbance	Regression equation
60	0.476	
120	0.480	y = 0,0002x + 0,4568
180	0.487	R^2 = 0,9643
240	0.506	
300	0.509	

The standard solution used in the measurement of antioxidant activities was Trolox. Trolox is commonly used as a positive control solution in antioxidant detection. Trolox acts as a vitamin E analog, a powerful antioxidant (Muflihunna et al., 2014). Trolox is the liquid form of vitamin E (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) that is widely used as a standard reference in antioxidant activity measurement, including FRAP. In the FRAP testing, Trolox acts as an electron donor to alter ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+}) that forms a blue-colored complex with TPTZ (2,4,6-tripyridyl-s-triazine). Moreover, Trolox can be used to measure antioxidant activities of a sample (Syarif et al., 2015).

The FRAP value was stated in µmol Trolox Equivalent/g or µmol TE/g. Each replication of antioxidant activity was stated as Trolox equivalent (TE). Trolox equivalent is a term used to measure antioxidant activities of a compound compared with Trolox, as a synthetic analog of vitamin E (tocopherol).

Table 4. The FRAP values of tenggulun *(Protium javanicum* Burm.fil) leaves extract

Replication	FRAP value (µmol TE/g)
1	0,009128
2	0,008688
3	0,008088
Mean ± SD	0,008634 ± $4,3x10^{-4}$

The FRAP values listed on table 4 resulted in the mean value of the tenggulun leaves extract to be 0,008634 µmol TE/g, meaning every gram of the extract was equivalent to 0,008634 mg of trolox. The value indicated that the antioxidant activity of tenggulun leaves were much inferior compared to Trolox. The antioxidant activity of 0,008634 µmol TE/g showed the ability to reduce Fe (III) into Fe (II) to be equivalent to Trolox. The low value of antioxidant activity showed that the sample had a relatively lower ability to reduce Fe (III) into Fe (II). The antioxidant activity of 0,008634 µmol TE/g is expressed to be a very low value. This antioxidant activity corresponds to the flavonoids and polyphenol as the phytochemical screening confirmed.

Thus, the low antioxidant activities in tenggulun leaves were influenced by the chemical compounds like flavonoid and polyphenol. The chemical compounds contained were acting as natural antioxidants responsible for antioxidant activities in tenggulun leaves. Although tenggulun leaves relatively low antioxidant activities, their potential utilization in cosmetics remains relevant. Currently, cosmetic industries are increasingly emphasizing innovative technologies and seeking alternative raw material sources, especially of plant origin, because they are of high biological value and environmentally friendly (Harhaun et al., 2020). Limited antioxidant activity does not necessarily diminish their functional value, as the bioactive compounds they contain can still play a role in slowing oxidative processes. In cosmetics term, aforementioned oxidative process might manifest in skin-aging and hyperpigmentation, discoloration and might lead to skin melanogenesis (Mapoung et al., 2021). Moreover, tenggulun leaves may be employed not only as active ingredients with protective functions against oxidative damage to the skin but also as excipients serving as natural preservatives in cosmetic formulations. Due to the fact that natural antioxidants have the ability to inhibit the processes of oxidation and the growth of microorganisms, including many pathogenic ones, e.g., *Salmonella* spp. and *Escherichia coli* they are more and more often used as preservatives in food product or as packaging ingredients in nutraceutical product. Therefore, despite having non-dominant antioxidant activity that is highly dependent on environmental factors, these medicinal plants still hold significant applicative prospects in the development of safer and more sustainable natural-based cosmetic products (Flieger et al., 2021).

CONCLUSION

Based on the antioxidant activity measurement in the tenggulun (*Protium javanicum* Burm.fil) leaves extract using the FRAP method and Trolox standard solution, the antioxidant activity of 0,0086344±0,00043 µmol TE/g was obtained. Tenggulun leaves has the potential to be developed

as both an active pharmaceutical ingredient and excipient in cosmetics either as protector against oxidative skin-damage and natural preservatives.

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