

Jurnal Farmasi Sains dan Praktis

(JFSP)

http://journal.unimma.ac.id/index.php/pharmacy



SUN PROTECTION FACTOR (SPF) VALUE AND PHYSICAL PROPERTIES OF PURIFIED GAMBIER GEL PREPARATION

Muhammad Asrofi Faizin¹, Purwanto²

¹Undergraduate Student, Faculty of Pharmacy, Universitas Gadjah Mada, Sleman 55281, Indonesia ²Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Sleman 55281, Indonesia

purwanto_fa@ugm.ac.id

https://doi.org/10.31603/pharmacy.v10i1.10208

Article info:			
Submitted	: 10-09-2023		
Revised	: 30-12-2023		
Accepted	: 26-01-2024		



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Publisher: Universitas Muhammadiyah Magelang

ABSTRACT Gambier is an export product from Sumatera, Indonesia, that can be purified and utilized in cosmetic preparations. The content of phenolic and flavonoid compounds of purified gambier has a potential skin protection against UV rays. The chemical content of extract is closely related to the polarity of solvent, so this study aims to analyze the effect of ethanol concentration as solvent in gambier purification on phenolic content, flavonoids, and SPF value. The ethanol concentrations for this purpose were 0%, 25%, 50%, 75% and 96%. Total phenolic and flavonoid contents were analyzed by colorimetric method, whereas the SPF value of gel was measured by UV spectrophotometer. The gel preparation was evaluated for its physical properties including organoleptic test, spreadability, adhesiveness, viscosity, pH and stability. Statistical analysis performed with one-way ANOVA at 95% confidence level. As the results, ethanol concentration significantly influenced phenolic, flavonoid content and SPF values. The highest phenolic content was obtained in purification by 50% ethanol with a value of 757.2 ± 13.1 mg GAE/g, while highest flavonoid content was achieved in 96% ethanol at 5.18 ± 0.21 mg QE/g. Ethanol concentration with highest SPF value was 96% at 27.07 \pm 0.33. In the gambier gel formulation with 0.2% dose had an SPF value of 6.60 \pm 0.58. The gel has good homogeneity, viscosity, and pH for cosmetic preparations but poor spreadability. The stability of the gel formulation changed after accelerated stability testing for 4 weeks.

Keywords: Flavonoid; Gambier; Phenolic; Purification; SPF

1. INTRODUCTION

Indonesia is a tropical country with high-intensity UV rays which is caused high index category of ultraviolet index. UV rays has negative effects on the skin such as loss of elasticity, fine lines, wrinkles, and premature ageing (Ansary et al., 2021). To protect our skin from exposure to UV radiation, an application of sunscreen preparations is one of the effective ways. For this purpose, FDA recommended a sunscreen preparation with the SPF value of more than 15 and repeated application at least every two hours (FDA, 2022)

The active substances in sunscreen preparations are divided into two types, that are inorganic and organic compounds. Unfortunately, inorganic materials have a risk of being absorbed into certain layers of skin because they are often made in nanoparticles for aesthetic reasons (Wang & Wang, 2014). On the other hand, the active substances of organic sunscreens need to be combined to improve their protection spectrum. However, the combination of organic compounds for sunscreens is restricted by regulatory agencies due to the risk of incompatibility (Ngoc et al., 2019).

Natural compounds can be an effective alternative as sunscreen agents because they generally consist of a mixture of various compounds that can absorb UV rays. Phenolic groups and their derivatives are chemical compounds with aromatic and chromophore groups which have

potential as UV absorbers. Polyphenols are coloured compounds which can absorb UV B radiation as well as some wavelengths of UV A and UV C. Polyphenols can protect our skin from UV radiation when applied topically (Hashemi et al., 2019). Studies conducted by Stevanato et al. (2014) showed that phenolic compounds such as catechins, quercetin, rutin, kaempferol and other flavonoid compounds have absorption at UV wavelengths (290-400 nm) depending on their structure and mass.

Gambier is a popular export commodity in Sumatera region because it is widely used by other countries as an ingredient for medicine, cosmetics, food, and leather tanning (Hernani et al., 2020). Gambier is the dried extract of the leaves of *Uncaria gambir* (Hunter) *Roxb*. from *Rubiaceae* familiy (Indonesia Herbal Pharmacopoeia II, 2017). Gambier contains free phenolic compounds and flavonoids such as catechins, quercetin, kaempferol, rutin and tannin (Munggari et al., 2022). Research conducted by Winarti et al. (2022) showed that gambier has UV protection activity showed by the SPF value in lotion preparations containing its extract. The SPF produced value is proportional to the amount of gambier extract added to the lotion preparation.

The gambier produced by the traditional way has variations in catechin content ranging form 40-60% due to differences in plant sources and processing (Hernani et al., 2020). Therefore, it is necessary to purify in order to increase the chemical content and remove unnecessary substances such as cellulose. The process can be done by water as solvents or in combination with organic solvents such as ethanol (Rauf et al., 2010). Purification is a process after extraction to separate a specific or group of compounds from others substances in extract that may contain undesired activities or inert constituents (Xiao et al., 2013). The concentration of ethanol in water will affect the polarity of the solvent mixture which determines the solubility of diluted secondary metabolites of an herb. Solvent selection is one of several important aspects that need to be considered in the purification process to obtain suitable extraction conditions (Mauricino & Juliana, 2013). The effect of ethanol concentration on several responses in the gambier purification process is the focus of this study. The obtained gambier from the purification process was made into gel preparation to determine the SPF value, physical properties, and stability of the preparation.

2. METHODS

2.1. Materials

The instruments used in this research are Mettler Toledo analytical balance (Ohio, United States), BenchTop Freeze Dryer (Tokyo, Japan), quartz cuvette 1 cm, Hitachi UH5300 Spectrophotometer UV-Vis (Tokyo, Japan), and Merck pH paper (Darmstadt, German). Materials used include gambier with various forms obtained from Beringharjo Market, Yogyakarta; ethanol 96%; distilled water; methanol pro analysis; ethanol pro analysis (Smartlab); Folin-Ciocalteu reagent; sodium hydroxide, anhydrous sodium acetate (Merck), gallic acid (Woko Pure Chem), aluminum chloride hexahydrate, quercetin (Sigma-Aldrich), carbopol 940, propylene glycol, propylparaben, methylparaben, and triethanolamine.

2.2. Methods

2.2.1. Purification of Gambier

The purification of gambier involves extracting the sifted gambier powder with a mesh size of 40 by ethanol as solvents with various concentrations for 3 days at room temperature. The ratio of solvent to sample used was 1:5. Maceration was repeated once by 1:3 solvent ratio for 1 day. The filtrate was then evaporated by a water bath system and subsequently dried by freeze-drying technique. The ethanol concentrations for purification are 0%, 25%, 50%, 75%, and 96%.

2.2.2. Determination of Phenolic Content and Flavonoid Content

The determination of phenolic content in purified gambier was carried out by Folin-Ciocalteu method following the procedures outlined in the Indonesian Herbal Pharmacopoeia II (2017), with several changes. Purified gambier was diluted with methanol to obtain a solution with a concentration of 0.1 mg/mL. A standard curve was generated by diluting gallic acid to create a series of dilution solutions at concentrations of 100, 70, 50, 30, 15, and 5 μ g/mL. A volume of 1.0 mL of the sample solution was taken and mixed with 5.0 mL of 7.5% Folin-Ciocalteu reagent. The mixture was incubated for 8 minutes, added by 4.0 mL of 1% NaOH, and then reincubated for 1 hour. The sample was placed in a cuvette, and its absorbance was measured alongside a blank solution at a maximum wavelength of 730 nm. The total flavonoid content of purified gambier was determined following the procedures of the Indonesian Herbal Pharmacopoeia II (2017). A dilution series of quercetin standard was prepared by concentrations of 100, 75, 50, 25, and 5 μ g/mL. Gambier samples were diluted with ethanol to get concentration of 10 mg/mL. A volume of 0.5 mL of both sample and reference solutions were taken and combined with 1.5 mL of ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M sodium acetate, and 2.8 mL of distilled water. The solution was shaken and left at room temperature for 30 minutes. The absorbance of all solutions, including samples, reference standard, and blanks was measured at a maximum wavelength of 438.0 nm.

2.2.3. SPF Value Measurement of Purified Gambier

The measurement of the SPF value of purified gambier was conducted following the procedure carried out by Dutra et al., (2004) with modifications to the sample dilution. The sample was diluted by ethanol to achieve a solution concentration of 2 mg/mL. The absorbance of the solution was measured at 5 nm intervals within the wavelength range of 290 nm-320 nm by UV-Vis's spectrophotometer. Each data point was measured three times. The SPF value was calculated by the equation formulated by Mansur et al. (1986), as following Eq. (1).

$SPF = CF \times \Sigma_{290^{\circ}320} E(\lambda) \times I(\lambda) \times Abs(\lambda)$ (1)

Where: CF: Correction Factor = 10; EE: Erythema Effect Spectrum; I: Intensity of Solar Spectrum; Abs: Absorbance of Sunscreen Formulation

The constant value of EE x I was obtained from Sayre et al. (1979), as seen in Table 1.

Wavelength (nm)	EE x I
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180
Total	1,0000

Table 1. Constants EE x I at each wavelength

2.2.4. Formulation of Purified Gambier Gel

Purified gambier with the highest SPF value was formulated into a gel preparation at a dosage of 0.2%. This powder dosage was chosen as it provides medium-level protection at a concentration of 2 mg/mL. The gel formulation follows the guidelines of Rina et al. (2019), with modifications to the type of carbopol as specified in Table 2.

Carbopol was dispersed in 50 mL of water and stirred homogeneously. The gel base was kept at room temperature for 1 night to fully expanded. Gambier powder was dissolved in 40 mL of water and stirred until fully dissolved. Separately, methylparaben and propylparaben were dissolved in propylene glycol and sonicated to help its solubilization. All components including the active ingredient and preservatives were added into the gel base and stirred to achieve a homogenous form. Finally, distilled water was added and stirred to reach a total amount of 100

g. Triethanolamine was added to adjust the pH value of the dosage form ranging from pH 5-6. After we got a homogeneous gel dosage form, the SPF value was measured by spectrophotometric method.

Table 2. Furthed Gambler Ger Formulation				
No	Gel Ingredients	Composition (w/w)		
1	Carbopol 940	1.00%		
2	Propylene glycol	5.00%		
3	Purified gambier	0.2%		
4	Methylparaben	0.18%		
5	Propylparaben	0.02%		
6	Triethanolamine	q.s		
7	Distilled Water	Added up to 100 g		

Table 2. Purified Gambier Gel Formulation

2.2.5. Assay SPF value and physical properties purified gambier gel

As SPF value measurement, approximately 1000 mg of gel was dissolved by 10 mL of ethanol p.a and assisted by sonication for 5 minutes. The resulting solution was separated from the insoluble solids by centrifugation at a speed of 8000 rpm for 5 minutes. The absorbance of the sample was measured at 5 nm intervals within the wavelength ranging from 290 nm-320 nm. Each data point was read three times (n=3), while the SPF value was calculated by the equation formulated by Mansur et al., (1986).

In this research, we also analyzed the physical properties of gel to assess the quality of the preparation. Organoleptic testing includes colour, odour, homogeneity, and texture were observed in the purified gambier gel preparation. The homogeneity was assessed by applying the gel onto two transparent glass plates to observe the presence of coarse particles or clumps within the formulation. The gel was considered homogeneous if there was no precipitate or clumps which were observed. The viscosity of the gel preparation was determined by a Brookfield Viscometer with the parameters of size 7 spindle, speed of 100 rpm, and measurement duration of 60 seconds. The spreadability of the gel was assessed by weighing 500 mg of gel onto a scaled round glass plate, then covered by second glass with a known weight and left for one minute. An additional weight of 50 grams was added to the gel every minute to be a total weight of 250 grams was reached. The spreadability of the gel was measured by calculating the average diameter of its spreading.

The adhesion test of the gel formulation was performed by weighing a 100 mg of gel onto an object glass plate and another glass plate was added on top of the gel. The glass plates holding the gel were subjected to a weight of 1 kg for 5 minutes and then placed on an adhesion testing apparatus connected to a weight of 80 grams. The time taken for the two glass plates to separate or detach since the weight was applied was recorded as an adhesion testing parameter. In the other analysis of gel, the pH of the gel preparation was measured by pH paper. Colour changes in the paper were observed to determine the pH range of the preparation based on the colour indicators specified on the packaging (Tambunan & Sulaiman, 2018).

The stability of the purified gambier gel preparation was evaluated following the accelerated testing conditions from WHO (1996) with the study duration shortened to four weeks. The gel was stored in a climatic chamber at a humidity of $75 \pm 5\%$ relative humidity (RH) and a temperature of 40 °C \pm 2 °C. The evaluation of the preparation's stability included organoleptic testing, spreadability, adhesion, and pH.

2.2.6. Statistical analysis

Data were analyzed using one-way ANOVA to evaluate the influence of ethanol concentration variation on total phenolic content, total flavonoid, and SPF value. Data that did not meet the assumptions of one-way ANOVA were replaced with Kruskal-Wallis analysis. Statistical analysis was performed using SPSS® software version 25 at a confidence level of 95%.

3. RESULTS AND DISCUSSION

3.1. Chemical Content

3.1.1. The Phenolic Content

The concentration of ethanol during the gambier purification process significantly influences its phenolic content, as depicted in Figure 1 (sig=0.011). Gambier contains a substantial amount of free phenolic compounds with carbon and aromatic groups that are better suited for extraction by a semi-polar solvent, such as ethanol. When the polarity of the solvent matches the nature of the compounds being extracted, more compounds will dissolve (Mauricino & Juliana, 2013). A concentration of 50% ethanol provides the highest phenolic content compared to other solvents, making it a suitable choice for gambier purification to achieve the highest total phenolic content. The findings of this study differed to Pambayun et al., (2007) because their research indicated that application of ethanol as a sole solvent resulted in a higher total phenolic content compared to an ethanol-water mixture (1:1), although the difference is not significant, as observed in this study.

3.1.2. The Flavonoid Content

The results of the analysis of total purified gambier flavonoids were shown in **Figure 1**. The total flavonoid content of gambier differs significantly due to the purification process by various ethanol concentrations (sig=0,000). Ethanol with high concentration produces correspondingly to high flavonoid content as well. This phenomenon occurs because gambier contains various types of flavonoid compounds, primarily catechins, as their main aglycone, which has low solubility in water (Kumar & Pandey, 2013).

3.2. SPF Value of Purified Gambier

The SPF value of purified gambier was measured to determine its protective capability against ultraviolet (UV) radiation. The purification process of gambier by various concentrations of ethanol had a significant impact on its chemical composition. Consequently, the SPF value of the purified gambier also exhibited significant differences at each ethanol concentration which was presented in **Figure 1** (*sig*=0,000). The SPF measurements were conducted on the prepared solutions by a spectrophotometer with an extract concentration of 2 mg/mL. This concentration was selected as it can provide a high SPF value without exceeding the sensitivity limits of the spectrophotometer. According to Yulianti et al. (2015), an absorbance limit of 4 is considered good for SPF measurements because absorbance values above 4 may made unstable data. Purified gambier at this concentration provided medium-level protection with an SPF value greater than 15 (European Communities, 2006). In this research, the highest SPF value did not correlate with its phenolic content. It was indicated that chemical compounds responsible for SPF value was not only a phenolic compound.

The purified gambier contains flavonoid aglycone compounds that are more soluble in ethanol due to their polarity nature. Flavonoid aglycone compounds are known to absorb UV radiation ranging from 275-295 nm and 300-330 nm, which is inferred as an effective UV protector. Flavonoids in the form of flavon, flavonols, and other flavonoid groups have the potential as UV radiation absorber.

3.3. The Evaluation of the Gel

3.3.1. Organoleptic Evaluation of Purified Gambier Gel

The gel had an orange colour because of the presence of phenolic compounds from purified gambier. The gel exhibited a soft texture and a slightly sticky feel due to its high viscosity. The distinct scent of the gel was a blend of the characteristic odor of carbopol and a slight gambier aroma. The addition of perfume was necessary to mask the natural scent of the gel. The gel showed good homogeneity without any visible solid particle on the two glass plates.



Figure 1. Effect of ethanol concentration to total phenolic contents, total flavonoid contents, and SPF value of extract

3.3.2. SPF Value of the Gel Formulation

The gel formulation containing 0.2% w/w purified gambier produced a SPF value of 6.60 \pm 0.58. This result was obtained from a gel formulation with 10 times dilution, which was ready for absorbance measurement by a spectrophotometer. The SPF value of the gel was lower than the purified gambier solution, highlighting the challenges of accurately representing SPF values through spectrophotometric measurement. Many factors influence SPF measurement through spectrophotometric methods, including concentration, excipients used, formulation pH, solvent selection, interactions between components, and other factors that can influence UV absorption (Faizin, 2023).

3.3.3. Physical Characteristics of the Gel Formulation

The physical properties of the gel formulation were measured and presented in Table 3. The viscosity of the gel met the requirements, but its value was relatively high, resulting in a thick and sticky consistency. High viscosity impacted the spreadingability of the gel, as greater viscosity leads to increased resistance to flow. Ideally, gel formulations should possess lower viscosity to ensure easy spreading. However, higher viscosity offers the advantage of improved adhesion. Optimizing the quantities of carbopol, propylene glycol, and TEA is necessary to achieve suitable viscosity levels. The pH of the gel formulation complied with sunscreen standards and was within the range of physiological skin pH (4-6), which is minimizing the potential for skin irritation caused by the product.

	Table 3. Physical Test Result of Gel				
No.	Physical Parameters	Test Results	Standard value		
1.	Viscosity	23.806±1137 cps	2000-50.000 cps (SNI, 1996)		
2.	Spreadability	4,6±0,3 cm	5-7 cm (SNI, 1987)		
3.	Adhesiveness	$2,64 \pm 0,38$ detik	>1 s (Lieberman <i>et al.</i> , 1996)		
4.	pH	5-6	4,5 – 8,0 (SNI, 1996)		

3.3.4. Stability of the Gel Formulation

Organoleptic observations indicated a change of color of gel formulation from orange in the first week to be a darker red hue in the second week. This colour change is attributed to the oxidation of catechin compounds in gambier, resulting in a transition from yellow to dark brown. Other organoleptic properties such as scent, texture, and homogeneity remained unchanged. While the adhesion of the gel formulation did not exhibit significant change, there was a decreasing trend, while the spreading ability showed a slight increase. The stability changes were shown at **Figure 2**. These changes were attributed to the characteristics of carbopol viscosity, which decreases after four weeks of storage. Gel formulations can absorb moisture which is leading to decreased viscosity and increased spreading diameter. The pH of the gel formulation remained stable throughout the four-week testing period, remaining within the 5-6 pH range. Longer stability studies are necessary to determine the gel ability to maintain its physical properties and efficacy during the self-life periode.



Figure 2. Stability changes of gel contains purified gambier

3.3.5. Overview Effect of Solvent Variations on Several Responses

The variation of solvent during the purification process of gambier has an impact on several responses such as phenolic content, flavonoid content, and SPF value. Additionally, the concentration of ethanol also affects the filtration process. The higher ethanol concentration makes it easier to separate solids from the filtrate because gambier contains polysaccharide compounds that can expand in water and clog the filter.

Ethanol at 75% could be a choice in the purification process of gambier because it produced a high SPF value, phenolic content, and flavonoid content, and eased in filtration. Although the highest response was obtained from 96% ethanol, it was more expensive and its response was not significantly different from 75% ethanol. However, further testing was needed for the selection of these two solvents because the industry will consider the solvent's recovery ability and the energy required for solvent removal.

4. CONCLUSION

The concentration of ethanol in the gambier purification process significantly influences the total phenolic content, total flavonoid content, and SPF value. The ethanol concentration that yields the highest total phenolic content is 50%, whereas the highest total flavonoid content and SPF value are achieved at an ethanol concentration of 96%. Ethanol 75% can be a suitable choice for gambier purification as it provides a response that is not significantly different from ethanol 96%, while also being more cost-effective. The SPF value of gel as low protection as a sunscreen. The purified gambier gel formulation meets the physical requirements for gel formulations, including organoleptic properties, viscosity, adhesion, and pH, but does not meet the spreading ability requirement. The gel formulation also experiences changes in stability, including colour, adhesion, and spreading ability.

5. ACKNOWLEDGMENT

We thank to Professor Endang and Dr. Andayana for helpful advice in this research so we could understand more in area of in-vitro test, natural product extraction and analytical method. Thank you very much also for Department of Pharmaceutical Biology and Department of Pharmaceutics which has been very helpful to give support and knowledge.

6. CONFLICT OF INTEREST

All authors declare no conflict of interest.

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