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Extraction of Gambier using the boiling method and antibacterial activity test

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Abstract

Background Gambier is a dry extract derived from the *Uncaria gambir* (Hunter) Roxb plant. Gambier can be processed by boiling, pressing, draining, moulding and drying.Gambier contains catechin compounds that have antibacterial activity.
Method In the research, gambier was extracted using the boiling method. Subsequently, the quality of the resulting gambier was tested based on the SNI 01-3391-2000 quality standard, and its antibacterial activity was evaluated against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.*
Results Based on the quality test of the gambier extract, it was found that the extract met the SNI 1 quality standard. The antibacterial tests showed that the gambier extract at concentrations of 10%, 15%, and 20% exhibited strong inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*. Meanwhile, against *Pseudomonas aeruginosa*, the extract at 10% concentration showed moderate inhibition, while at 15% and 20% concentrations, it showed strong inhibition**.**
Conclusion Gambier has tobe good antibacterial activity and potential to be developed into a natural ingredient product with potential as an antibacterial.

Keywords: Antibacterial, Catechin, Gambier

INTRODUCTION

Infectious diseases are often found in tropical areas such as Indonesia because of the dusty air, warm temperatures, and humidity, which allow microbes to thrive. These conditions are supported by poor sanitation conditions that make developing infectious diseases easier (1). Most infections in Indonesia are caused by the bacteria *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli* (2). Irrational use of antibiotics can increase cases of antibiotic resistance (Schneider, 2021). In addition, antibiotics are one of the drugs that have considerable side effects (3).

Plant utilization can be a source of compounds that have antibacterial activity (4). For this reason, various plants that have antibacterial properties are quite widely studied. Utilizing natural materials as medicine is the right thing to do in supporting life. Plants and herbs are natural ingredients with several advantages, including fewer side effects than synthetic drugs.

Indonesia is one of the largest archipelagic countries in the world, possessing an exceptionally high diversity of plant biodiversity. Among this rich flora, many plant species have medicinal properties and have been used in traditional medicine for generations, based on local knowledge and experience (5). Currently,

approximately 7,000 out of 30,000 plant species in Indonesia are believed to have potential as medicinal resources (6).

One of the plants that has potential as an antibacterial agent is gambir(7). Gambir grows in tropical regions and belongs to the Rubiaceae family. It is widely found in various areas, including the islands of Sumatra and Kalimantan (8). Indonesia is a global producer of gambier, controlling 80 percent of the world's gambier market share(9).

Gambier is a dry extract derived from the *Uncaria gambir* (Hunter) Roxb. plant. Gambier can be processed by boiling, pressing, draining, moulding and drying (10).

Gambier is a type of herbal plant known in Indonesia for a long time as an additional ingredient in betel chewing and as an additional substance in traditional herbal medicine. Also, gambier has been used to treat diarrhoea, sore throat, scabs, burns and healing wounds (11).

Gambier contains several components, such as catechin, epicatechin, procyanidin B1, procyanidin B3, gambir A1 and gambir (12).

Catechins in gambier play an important role in various activities, including antioxidant (13), anticancer(14)(15), and antibacterial effects (16).

Catechins, present in high concentrations, demonstrate potent antibacterial activity by targeting and inhibiting the biosynthesis of peptidoglycan, an essential polymer that provides structural integrity to bacterial cell walls. This inhibition compromises bacterial cell wall formation, leading to cell lysis and death, highlighting catechins' potential as natural antimicrobial agents (17).

The most widely utilized chemical content of gambier is catechin(10). Catechins are flavonoid compounds with antibacterial activity (18). This study aims to evaluate the antibacterial activity of Gambier extract, obtained through the boiling method, against *Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli*

TOOL

The tools used in this research are a grinder, UV/VIS Spectrophotometer (T70), vaporizer cup, Gooch cup, mortar and pestle crucible, erlenmeyer, Beaker glass, measuring flask, tweezers, spatel, digital scale, funnel, tissue, aluminum foil, pot, beaker glass, and autoclave.

METHOD

Quality parameter testing of Gambir in accordance with the Indonesian National Standard (SNI) for Gambir (2000) (19) and antibacterial activity testing using the agar diffusion method (20).

A. Simplisia preparation

Two kilograms of gambier leaves steamed using a steamer for ± 30 hours. Next, the gambier leaves were removed and cooled for a few minutes, then dried in an oven at 60˚ C for 3 hours.

B. Gambier extraction

Put 300 grams of gambier leaves simplisia into a pot and add 3 L distilled water. Heat until boiling and leave it boiling for 1 hour. Filter the results of the decoction, put it in a bottle, and let it stand for 24 hours in the refrigerator. Take the sediment out and dry it in an oven for 24 hours at 50oC.

C. Gambir quality inspection

1. Organoleptic (19)

The organoleptic examination is done in shape, color, taste, and smell.

2. Water content (21)

Weigh two grams of sample and put it into a crust that has a constant weight. Dry it in the oven at 105°C for two hours. Leave the desiccator to room temperature, and then the crucible is weighed. Do the procedure until the weight is constant. The water content is determined with the formula:

$$\%\text{Water content} = \frac{(B-A)-(C-A)}{(B-A)} \text{X} 100\%$$

Description:

A = weight of empty porcelain crucible

B = weight of porcelain crucible + sample before drying

C = weight of porcelain crucible + dried sample

3. Ash content (19)

Weigh 2 grams of the ground sample and place it into a preheated and tared silicate crucible, ensuring an even distribution. Slowly ignite the sample until all charcoal is completely burned off. Allow the crucible to cool and then weigh it. If charcoal remains, add hot water and filter the solution using ash-free filter paper. Ignite both the residue and filter paper in the same crucible. Transfer the filtrate into the crucible, evaporate the liquid, and ignite until a constant weight is achieved. Finally, weigh the crucible and calculate the ash content of the air-dried material.

$$\%\ ash\ content = \frac{C - A}{B - A} \times 100\%$$

Description:
A = Weight of the empty porcelain crucible
B = Weight of the porcelain crucible with the sample
C = Weight of the porcelain crucible with the incinerated sample (ash

4. Determination of Water Insoluble Materials (19).

Weigh ± 1 g of the dry (water-insoluble) sample that has been pulverized into a 200 ml evaporator cup containing 100 ml of water; heat the mixture to boiling, then filter it using a Gooch cup known to be tight; dry the Gooch cup containing the residue in an oven at 105 ° C ± 1 ° C for 1 hour; cool in a desiccator for 30 minutes and weigh until the weight is constant.

$$\%\ water\ insoluble = \frac{W2 - W}{W1}\ x\ 100\%$$

Description:
W = Weight of dry sample material
W1 = Weight of Gooch crucible
W2 = Weight of water insoluble residue

5. Determination of Alcohol Insolubility (19)

Weigh ± 1 g of crushed dry sample (water-soluble) into a 200 ml goblet containing 100 ml of alcohol; close the Erlenmeyer with a cotton-tipped cork stopper; heat the mixture to boiling then filter it using a Gooch crucible of known weight; dry the Gooch crucible containing the residue in a 105°C ± 1°C oven for 1 hour; cool in a desiccator for 30 minutes and weigh until the weight is constant.

$$\%\ alcohol\ insoluble = \frac{W2 - W}{W1} \times 100\%$$

Description:
W = Weight of dry sample material
W1 = Weight of Gooch crucible
W2 = Weight of alcohol insoluble residue

6. Determination of Catechin Content (19)

The stages carried out in determining catechin levels are:

1. Preparation of Catechin Standard: dry the catechin standard in an oven at 105°C for 3 hours.
2. Preparation of Gambier Sample: grind the gambier sample and dry the gambier in the oven at 105°C for 3 hours until weight loss of 15% - 17%.
3. Preparation of the Standard Solution: Accurately weigh 10 mg of the dried catechin standard (Ws mg) and transfer it into a 10 ml volumetric flask. Dissolve and dilute the sample using ethyl acetate to obtain solution A. Heat solution A in a water bath for 5 minutes to ensure homogeneity. Then, precisely pipette 2 ml of solution A into a 100 ml screw-top Erlenmeyer flask, add 50 ml of ethyl acetate (solution B), and heat the mixture in a water bath for another 5 minutes. Solution B is now ready for analysis.
4. Preparation of Sample Solution: Accurately weigh 10 mg of dried gambier sample (W mg) and transfer it into a 10 ml volumetric flask. Dissolve and dilute with ethyl acetate up to the mark to obtain solution C. Heat solution C in a water bath for 5 minutes, then filter it. Discard the first 1.5 ml of filtrate and continue the

filtration process. Carefully pipette 2 ml of the filtrate from solution C into a 100 ml screw-top Erlenmeyer flask, then add 50 ml of ethyl acetate (solution D). Heat solution D in a water bath for 5 minutes. Solution D is now ready for analysis.

5. Solution Measurement: The solution was analyzed using an Ultraviolet Spectrophotometer at wavelengths of 279 nm and 300 nm. The procedure involved measuring the absorbance of the blank solution (ethyl acetate), which should be 0, followed by measuring the absorbance of the standard solution at 279 nm (Ec). Finally, the absorbance of the sample solution was recorded at the same wavelength.

$$\%catechin = \frac{Et\ 279}{Ec\ 279}\ x\ \frac{Ws}{w} \times 100\%$$

Description:
Et 279 = absorbance/absorption of sample solution at a wavelength of 279 mm;
Ec 279 = absorbance of standard solution at a wavelength of 279 mm;
Ws = weight of standard catechin, expressed in mg;
W = weight of gambier sample, expressed in mg.

D. Antibacterial activity testing

Sterilization of tools

All tools used in the antibacterial activity test were washed and dried. The equipment was sterilized in an autoclave at 121°C for 15 minutes (20)

1. Preparation of NA agar medium

A total of 5 grams of nutrient agar (NA) was dissolved with 250 mL of distilled water in an erlenmeyer and heated on a hotplate using a stirring rod until a clear solution was formed. The media was sterilized in an autoclave at 121ºC for 15 minutes. NA is then put into several test tubes with a predetermined amount; the tube that contains the agar is placed on a slope of 30-400. Let it harden. (20).

2. Preparation of Gambier test solution

A total of 3 grams of gambier was placed into a 10 mL volumetric flask and dissolved in DMSO up to the mark, resulting in a 30% mother solution. From this stock solution, dilutions of 10%, 15%, and 20% (b/v) were prepared. A sterilized agar medium was poured into Petri dishes, and paper discs were immersed in the extract solution. The discs were then removed, placed on bacterial cultures, and incubated at 37°C for 24 hours. The negative control used was 10 µL of DMSO, while the positive control was a 30 µg/mL chloramphenicol antibiotic disc. After incubation, the inhibition zone diameter around each disc was measured using a caliper. The antibacterial activity was then classified based on the inhibition zone diameters, as outlined in Table 1.

Table 1. Classification of Antibacterial Activity Strength (22).

Inhibition Diameter	Strength of Antibacterial Activity
< 5 mm	Weak
6-10 mm	Medium
11-20 mm	Strong
21 > mm	Very Strong

DISCUSSION

Gambier leaves are processed in several stages so that they become dry simplisia. Gambier leaf simplisia powder is obtained by steaming fresh gambier leaves directly for 1 hour to inactivate polyphenol oxidase compounds in gambier leaves because polyphenol oxidase compounds can damage the quality of gambier. After steaming, the gambier leaves are then oven at 60°C for 3 hours to dry to prevent mould growth on the gambier leaves. After the oven, the dried gambier leaves are pulverized using a grinder.

Next, weigh the simplisia as much as 300 grams, put it in a pot, add 3 L of water, and let it boil for 1 hour. After boiling, filter and separate the solvent from the pulp. Put the filtrate into a glass bottle and allow it to

stand in the refrigerator for 24 hours. The goal is to accelerate sediment formation because gambier extract quickly precipitates at cold temperatures and dissolves at hot temperatures. After 24 hours, the gambier extract was separated by filtering. The precipitate obtained was then oven-dried at 60˚C. From the extraction process, an 18% yield was obtained.

Table 2. Quality inspection results of gambier extract based on SNI gambier

Parameters	SNI Quality 1	SNI Quality 2	Result of evaluation
Catechin (%)	Minimum 60	Minimum 50	88,2
Water Content (%)	Maximum 14	Maximum 16	6,92
Water Insoluble Materials (%)	Maximum 7	Maximum 10	2,22
Alcohol Insoluble Materials (%)	Maximum 12	Maximum 16	2,76
ash content (%)	Maximum 5	Maximum 5	3,91

The obtained Gambier is then tested for quality according to SNI Gambier (01-3391-2000), which includes organoleptic, ash content, water content, water-insoluble material, non-alcoholic material and catechin content. Organoleptic examination showed that the Gambier obtained was yellowish, slightly rough powder with a distinctive gambier odour, astringent taste, and slightly bitter. The results of the examination of ash content in gambier extract obtained a value of 3.91%. Examination of water content in gambier extract obtained a water content value of 6.53%. Examination of insoluble materials in water gambier extract obtained a value of 2.26%, while insoluble materials in alcohol gambier extract obtained a value of 7.69%. The determination of gambier catechin content aims to determine the catechin content in gambier dry extract. In the test, the catechin content was found to be 88.20%. From all tests, it can be concluded that Gambier obtained by boiling meets quality 1 in SNI Gambier (01-3391-2000); the results can be seen in Table 2.

The results of the antibacterial activity of Gambier against *Staphylococcus aureus* obtained the following data: 10%, 15%, and 20% concentrations are 12.53 mm, 14.88 mm and 15.93 mm. The results of testing the antibacterial activity of Gambier on *Escherichia coli* obtained the following data: 10%, 15%, and 20% concentrations are 12.18 mm, 12.44 mm and 12.6 mm. The results of testing the antibacterial activity of Gambier on *Pseudomonas aeruginosa* obtained data as follows: 10%, 15%, and 20% concentrations are 9.35 mm, 10.30 mm and 12.66 mm (table 3).

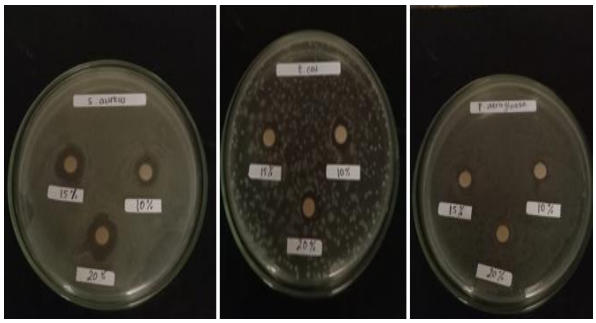
Table 3. Antibacterial activity of gambir extract

Sample	*S. aureus*	*E. coli*	*P.aeruginosa*
Catechin 10%	12,53 mm	12,18 mm	9,35 mm
Catechin 15%	14,88 mm	12,44 mm	10,3 mm
Catechin 20%	15,93 mm	12,66 mm	12,32 mm
Positive control	35,55 mm	33,75 mm	44,97 mm
Negative control	-	-	-

In the positive control using chloramphenicol, the results obtained on *Staphylococcus aureus* 35.55 mm, *Escherichia coli* 33.75 mm and *Pseudomonas aeruginosa* 44.97mm.

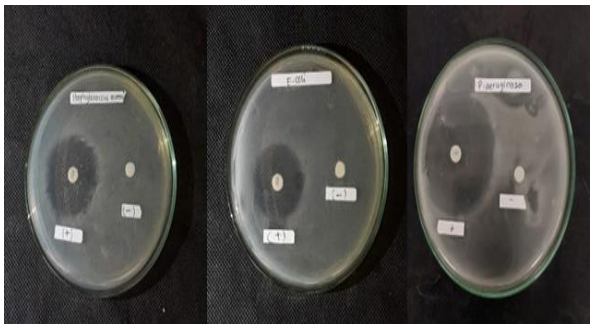
Based on the observed inhibition zone diameters, Gambier exhibited strong antibacterial activity against *S. aureus* and *E. coli* at all tested concentrations, as well as against *P.* aeruginosa at 15% and 20% concentrations. However, at 10% concentration, its inhibition against P. aeruginosa was moderate. *S. aureus* and *E. coli* are gram-positive bacteria, whereas *P. aeruginosa* is gram-negative. The findings

suggest that Gambier is more effective against gram-positive bacteria. This may be attributed to the structural complexity of gram-negative bacterial cell walls, which include an outer membrane and a cytoplasmic membrane separated by a cross-linked peptidoglycan layer, resulting in distinct physicochemical properties that may reduce susceptibility to antibacterial agents (23).



A B C

Figure 1. Antibacterial activity test of catechins at different concentrations against (a). *Staphylococcus aureus*, (b). *Escherichia coli* and, (c). *Pseudomonas aeruginosa bacteria*



A B C

Figure 2. Antibacterial Activity Test of positive and negative control comparators against (a). *Staphylococcus aureus*, (b) *Escherichia coli* and, (c) *Pseudomonas aeruginosa bacteria*.

In this study, it can be concluded that the higher the concentration of catechins, the higher the inhibition against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

CONCLUSION

According to the research, it can be concluded that gambier extracted by boiling meets quality 1 based on SNI gambier (01-3391-2000). Based on the results of the antibacterial activity test, it can be concluded that gambier extract exhibited strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Meanwhile, against *Pseudomonas aeruginosa*, the extract at a concentration of 10% showed moderate inhibitory activity, whereas at concentrations of 15% and 20%, it exhibited strong inhibition. Gambier has good potential to be developed as an antibacterial.

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