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# The Antibacterial Activity of Gambir Extract (Uncaria gambir (hunter ) Roxb ) Against Salmonella typhi

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**Abstract.**
Typhoid fever is still a severe issue in the world. The pharmacological therapy used for typhoid fever was chloramphenicol antibiotics. However, chloramphenicol resistance to Salmonella typhi is common, so finding alternative antibacterial drugs for typhoid fever is necessary. Gambir Extract was a natural ingredient often used in non-pharmacological alternative therapies. It contained secondary metabolites such as alkaloids, flavonoids, tannins, and saponins which are suspected to be antibacterial. This study aimed to identify the Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration values of Gambir Extract against Salmonella typhi. The antibacterial activity of the Gambir Extract against Salmonella typhi was screened using the well diffusion method. The MIC and MBC of the Gambir Extract were determined using the dilution method. Data were analyzed using the Kruskal-Wallis test and the Mann-Whitney test. The inhibition zone of the antibacterial activity of the Gambir Extract against Salmonella typhi was 19.22 mm and had a MIC value of 50% (0.5 g/ml). The statistical analysis results of the Kruskal-Wallis and the Mann-Whitney test obtained significance values of 0.007 and 0.025. Therefore, Gambir Extract cannot kill Salmonella typhi. Gambir Extract has antibacterial activity against Salmonella typhi with a MIC value of 0.5g/ml.

**Keywords:** Antibacterial, Gambir, Minimum Bactericidal Concentration (MBC), Minimum Inhibitory Concentration (MIC)

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## 1. Introduction

Typhoid fever is a bacterial infection that attacks the human digestive system caused by *Salmonella typhi*. Typhoid fever often occurs in areas with poor environmental sanitation caused by *S. typhi* [1] . Typhoid fever in the world reaches 11 to 20 million cases per year which causes around 128,000-161,000 deaths each year [2]. Typhoid fever cases mostly occur in Southeast Asia, South Asia, and Sub-Saharan Africa. Typhoid fever in Indonesia reaches a prevalence rate of 358-810/100,000 Indonesian population, of which 64% of typhoid fever infections occur in patients aged 3-19 years [3]. A case study in 2015 noted

that a city in South Kalimantan Province had 413 cases of typhoid fever that year. The Indonesian Research and Development Agency stated that South Kalimantan Province is in 8th place out of 14 Provinces which has the largest prevalence in Indonesia with a prevalence of typhoid fever of 1.95%.

At this time, most people use conventional antibiotics to treat typhoid fever. Drug therapy used as a means of choice for typhoid fever is the antibiotic chloramphenicol, but currently there is a lot of resistance to several antibiotics, one of which is the *S. typhi* resistance to the antibiotic chloramphenicol[4] . It is estimated that more than 20 million people have positive cultures for *S. typhi* and 220,000 deaths occur annually and most of these isolates are resistant to several antimicrobials[5] . In addition, the thing that really wants to be avoided at this time is resistance to antibiotics. Antibiotic resistance in various strains of bacteria that cause infection is a threat to public health[6] . An increase in the resistance of *S. typhi* to the antibiotic chloramphenicol was reported by 63.6% to 100% during the period 1996 to 2008 which remained constant until 2015[7] . The number of cases of antibiotic resistance has led to the development of research on new alternative antibacterial compounds from natural sources.

*The World Health Organization* (WHO) states that 80% of the world's population has used herbal medicines. This is due to the attractiveness of traditional medicine, which mainly comes from its natural properties, so it is considered safer and better tolerated than modern medicine. Based on research, the antibacterial activity of the methanol extract of the stem bark of *Rhizophora mucronata Poiret* against *Salmonella thypi, Lignières 1900 (Enterobacteriaceae: Gammaproteobacteria)* shows that the diameter of the inhibition zone produced by the methanol extract of the stem bark of *Rhizophora mucronata* with a concentration of 20%, 40 %, 60% and 80% (w/v) were classified as moderate (9.22 mm) to strong (13.78 mm), the greater the concentration, the greater the diameter of the inhibition zone, so that the methanol extract of *Rhizophora mucronata Poiret stem bark was* able to inhibit growth of *S. typhi* [8] . A research which tested the antibacterial activity of *jatropha curcas L.* and gambir leaf extracts against *Staphylococcus aureus* and *Escherichia coli.* can inhibit the growth of *Staphylococcus aureus* and *E. coli*, extracts of *Jatropha curcas L.* and gambir leaves are more effective in inhibiting *S.auerus* than *E.coli*[9] . The research about the antifungal activity of the ethanol extract of gambir leaves against *Candida albicans* showed that the ethanol extract of gambir leaves at all concentrations tested could inhibit the growth of the fungus *Candida*

*albicans* . Chloramphenicol as a positive control was more effective in inhibiting the growth of *Candida albicans* than gambir leaf extract[10] .

There have been many processes of using herbal plants carried out by Indonesian people, especially in remote areas of Indonesia. One of them is in the area of Central Kalimantan which uses gambier as a medicine for typhoid fever or what is commonly called typhoid, but there is no scientific evidence showing that gambier is efficacious as a medicine for typhus. Gambir is a typical Indonesian plant belonging to the *Rubiaceae tribe* . Based on phytochemical research, the main content of gambier is 74% catechins (flavonoids) which are polyphenolic compounds that have potential as antibacterial[11] .

Based on the description above, researchers are interested in conducting research to prove the antibacterial potential of gambier extract against *S. typhi.*

## 2. Methods

The research design used was the *Posttest-Only Control Group Design* by giving treatment to the experimental group and comparing the group to the control group. This research was conducted at the Laboratory of Pharmaceutical Microbiology, University of Sari Mulia. The population used in this study was gambir extract found throughout Indonesia. The sample used in this study was gambir obtained from UD. Juragan Jamu, City of Modinan, Gamping, Sleman, Special Region of Yogyakarta.

### 2.1. Tools and materials

The tools used in this study were *autoclaves, incubators, biological safety cabinets, hot plates, colony counters,* refrigerators, micropipette, Erlenmeyer, measuring cups, beakers, test tubes, test tube racks, analytical scales, dropper pipettes, stirring rods, petri dishes, ose needles, magnetic stirrer, tweezers, aluminum foil, filter paper, label calipers and spirits.

The materials used in this study were gambier extract*, S. typhi* bacteria culture , sterile aquadest, 96% ethanol, 0.9% NaCl, *Mueller Hinton Agar (MHA), Nutrient Broth* media (NB), *Dimethyl Sulfoxide* (DMSO) 10 %, Barium Chloride 1% (BaCl 1%) and Chloramphenicol.

## 2.2. Research procedure

## 2.3. Sample preparation

Gambir Simplisia as much as 500 grams into a glass jar and add 750 ml of 96% ethanol and leave it at room temperature for 24 hours. Macerated with 96% ethanol and stirred slowly, filtered every 24 hours to obtain the macerated extract. In this study, the gambier extract was prepared in different concentrations, namely 50 %, 75% and 100% w/v (g/10ml). The concentration is by weighing the extract 0.05 gram, 0.075 gram and 1 gram, then it is dissolved with 10% DMSO to a volume of 10 ml [12].

## 2.4. Sterilization of tools and materials

Tools and materials in this research will be sterilized using an autoclave at 121°C for 15 minutes.

## 2.5. Preparation of Mueller-Hinton Agar (MHA) Media and Nutrient Broth (NB) Media

MHA media was weighed, then dissolved with 200 ml of distilled water in an Erlenmeyer, then heated on a *hot plate* with the help of a *magnetic stirrer* until the homogeneous media became a clear yellow color. Once homogeneous, the media was sterilized using an autoclave for 15 minutes at 121°C. Sterile MHA media was poured into a petri dish of 25 ml, allowed to stand at room temperature until the media became solid and then stored at 4°C [13].

## 2.6. Making Positive Control and Negative Control

The positive control used was 200 µL of the antibiotic chloramphenicol which was made from 250 mg of chloramphenicol and then dissolved with 10% DMSO[14] .

The negative control used in this study was a 10% DMSO solution by dissolving DMSO using aquadest[15] .

## 2.7. Making Solution Mc. Farland no. 0.5

The composition of the Mc solution. Farland 0.5, namely 9.95 ml of H2SO4, 0.05 ml of 1 % $BaCl_2$ . Making a 1% $H_2SO_4$ solution by taking 1 ml of $H_2SO_4$ solution dissolved in 10 ml of distilled water and making a 1% $BaCl_2$ solution is done by weighing 0.1 $BaCl_2$ and dissolving it in 10 ml of distilled water. Mc.Farland's solution is prepared by taking 9.95 ml of $H_2SO_4$ mixed with 0.05 ml of $BaCl_2$solution [16].

## 2.8. Bacterial Rejuvenation

Media NB was weighed as much as 0.48 grams and dissolved with 60 ml of distilled water in Erlenmeyer, then heated the media on a hot plate until homogeneous. After that, cover the Erlenmeyer with cotton and then sterilize it by autoclaving for 15 minutes at 121ºC. then take the bacterial culture suspended in 60 ml of NB media, incubate at 37C for 24 hours[12] .

## 2.9. Preparation of Test Bacterial Suspensions

In this research, the preparation of the test bacterial suspension was carried out after the *S. typhi* had been rejuvenated for 24 hours in the incubator. *S. typhi* were taken 1 ml then suspended in 9 ml of 0.9% sterile NaCl solution and homogenized, then the turbidity was uniform using the standard Mc. Farland 0.5[12]

## 2.10. Antibacterial Screening

Antibacterial activity screening test of gambier extract was carried out on *S. typhi* µ using the well diffusion method. The bacterial *suspension that had been made was inoculated on MHA media with a cotton swab* , then incubated for 10 minutes, after which the wells were prepared with the help of a sterile *cork borer* (6 mm in diameter). In each well, the respective test concentrations of the gambir extract used were 50%, 75% and 100%. Positive control and negative control (DMSO 10%). The petri dishes were then incubated for 24 hours at 37ºC. The inhibition formed by the presence of a clear zone by observing the diameter of the inhibition zone around the well is measured using a caliper [12].

### 2.11. S. typhi Antibacterial Activity Test

Antibacterial activity test of Gambir extract against *S. typhi was tested using the Minimum Inhibitory Concentration* (MIC) and *Minimum Bactericidal Concentration* (MBC) liquid dilution methods . In this method, using 9 sterile test tubes aseptically by inserting NB media. After that, gambier extract was added to each test tube with a concentration of 50, 75 and 100%, then each tube was added to a bacterial suspension adjusted to the 0.5 Mc Farland standard, then incubated at 37◦C for 18-24 hours. Observe by looking at the turbidity (no bacterial growth ) and clarity (no bacterial growth) compared to chloramphenicol (positive control) and DMSO 10% (negative control). The lowest concentration that can inhibit bacterial growth is called the MIC[12] .

Then to find out *the* MBC, all the tubes used for the determination of MIC where there was no bacterial growth, 200 µL was taken using a micropipette from the suspension, then added to a test tube containing sterile MHA media, after which it was incubated at 37°C for 18-24 hours with three replications. No or no bacterial growth was observed which indicated bacterial colonies in the media. MBC, namely the lowest concentration which indicates no growth of bacterial colonies in the media[12] .

### 2.12. Data analysis

In this study the data analysis using the SPSS program.

## 3. Results and Discussion

Gambir is a plant extract that contains secondary metabolites such as alkaloids, flavonoids, tannins, saponins. Alkaloid compounds are secondary metabolites that are capable of inhibiting bacterial growth by inhibiting the formation of cell walls, especially the constituent components of peptidoglycan, which results in cell death. Flavonoid compounds contain catechin compounds which are active substances from gambier and have benefits as antibacterial. The mechanism of action of flavonoids is to inhibit the function of cell membranes and energy metabolism of bacteria. Saponin compounds have a mechanism of action by interfering with the stability of the bacterial cell membrane, causing bacterial cell lysis. Tannins are secondary metabolites which are polymers of phenolic compounds that can inhibit bacterial growth. Phenolic compounds

work by coagulating or agglomerating the bacterial cytoplasm so that stable bonds are formed with bacterial proteins [17].

## 3.1. Antibacterial Activity Screening Test Results

In this research, the antibacterial activity test of gambir extract against *S. typhi* was carried out using a screening test for antibacterial activity as a first step to determine the potential antibacterial activity of gambir extract against *S. typhi*. The method used in the screening study for the antibacterial activity of gambir extract is the well diffusion method. This study also used the dilution method as a test for antibacterial activity. This dilution method was used to determine the MIC and MBC of gambir extract against *S. typhi* . In this study, the test material used was gambir extract and the solvent used to dissolve the gambir extract was 96% ethanol and 10 % DMSO. Chloramphenicol is an antibiotic that has a broad spectrum so it can inhibit gram-positive or gram-negative bacteria. Chloramphenicol is also a type of antibiotic used in the treatment of typhoid fever and is the first choice antibiotic given for typhoid fever. So in this study chloramphenicol was used as a positive control and the bacteria used in this study were *S. typhi*, for this reason chloramphenicol is the right antibiotic to use [18].

The results of the screening test for antibacterial activity in this study showed that at a concentration of 50% gambir extract had an inhibition zone diameter of 17.73 mm and provided the smallest inhibiting effect on the growth of *S. typhi*. Then the diameter of the highest inhibition zone is at a concentration of 100% which has a value of inhibition zone diameter of 19.22 mm . The results of the average diameter of the inhibition zone obtained from the screening test for antibacterial activity are included in the *intermediate inhibition zone category* , because the inhibition zone category according to CLSI (2020) is divided into 3 categories, namely *susceptible* (zone diameter $\geq$ 20 mm), *intermediate category* (zone diameter 15-19 mm), and *resistant* category (zone diameter $\leq$ 14 mm). Based on the results of the antibacterial activity screening test, it is known that there is an inhibition zone so that the gambir extract used can be continued to test for antibacterial activity, namely MIC and MBC by testing using the dilution method.

The results of this study indicated that all concentrations of gambir extract had no killing power or MBC value against *S. typhi*. This is due to the growth of bacteria on MHA solid media at all concentrations of gambir extract. The same results were also found in gambir's antifungus research that has no MBC value for *S. typhi* [10].

Table 1: Observations of the screening test for antibacterial activity of gambir extract against *S. typhi* .

| No | Treatment | Replication | Diameter (mm) | Average |
|---|---|---|---|---|
| 1 | Gambir extract concentration 50% | I II III | 18,28 17,47 17,46 | 17,73 |
| 2 | Gambir extract concentration 75% | I II III | 20,16 16.95 18,69 | 18,6 |
| 3 | Gambir extract concentration 100% | I II III | 21,21 17.00 19.45 | 19,22 |
| 4 | Positive control (Chloramphenicol) | I II III | 30,16 37,69 37,69 | 35,18 |
| 5 | Negative control (DMSO 10%) | I II III | 0.0 0.0 0.0 | 0.0 |

## 3.2. MIC and MBC Test Results

Research on antibacterial activity test of gambir extract against *S. typhi* has never been done before. Based on the results of research on the antibacterial activity of gambir extract it has an inhibitory power or value of 50% (0.5 g/ml) MIC against *S. typhi*. But gambier extract has no killing power or MBC value against *S. typhi,* due to the complex cell wall structure of *S. typhi* , even higher concentrations are needed to kill *S. typhi*. The structure of gram-negative bacteria is more complex so that it is difficult for antibacterial substances to penetrate into bacterial cells so it is not easy to inhibit bacteria [20]. There are three layers of the cell wall of gram-negative bacteria which have a lipid content ranging from 11-12% which makes it more difficult for antibacterial substances to enter the bacterial cell so that the ability to inhibit gram-negative bacteria is lower [21]. The inhibitory ability of gambir extract is due to the presence of secondary metabolites such as alkaloids, flavonoids, tannins and saponins which have antibacterial properties [17] . Flavonoid compounds in gambir can inhibit bacterial growth due to the presence of catechin compounds which act as antibacterials. The higher the concentration of the gambir extract, the higher the content of the active substances of flavonoids which are derivatives of catechins in it so that the antibacterial activity will be greater and vice versa the lower the concentration of the gambir extract, the less the content of flavonoid substances which are catechin derivatives in it so that the antibacterial activity will decrease [10].

Based on the research results obtained, gambier extract , which has secondary metabolite compounds, is used as an antibacterial which is bacteriostatic according to

Table 2: Results of observation of the MIC test of gambir extract against *S. typhi* .

| No | Concentration Variation | Replication | | | p-value |
|---|---|---|---|---|---|
| | | I | II | III | |
| 1 | Gambir extract concentration 50% (0.5g/ml) | Clear | Clear | Clear | 0.007a _ 0.025b _ |
| 2 | Gambir extract concentration 75% (0.75 g/ml) | Clear | Clear | Clear | 0.007a _ 0.025b _ |
| 3 | Gambir extract concentration 100% (1 g/ml) | Clear | Clear | Clear | 0.007a _ 0.025b _ |
| 4 | Positive control (Chloramphenicol) | Clear | Clear | Clear | 0.007a _ |
| 5 | Negative control (DMSO 10%) | cloudy | cloudy | cloudy | 0.007a _ 0.025b _ |

Table 3: Observation results of the MBC test of gambir extract against *S. typhi* .

| No | Concentration Variation | Replication | | |
|---|---|---|---|---|
| | | I | II | III |
| 1 | Gambir extract concentration 50% (0.5g/ml) | growing colonies | growing colonies | growing colonies |
| 2 | Gambir extract concentration 75% (0.75 g/ml) | growing colonies | growing colonies | growing colonies |
| 3 | Gambir extract concentration 100% (1 g/ml) | growing colonies | growing colonies | growing colonies |
| 4 | Positive control (Chloramphenicol) | Do not grow colonies | Do not grow colonies | Do not grow colonies |
| 5 | Negative control (DMSO 10%) | growing colonies | growing colonies | growing colonies |

the results of determining MIC of gambir extract . The content of secondary metabolites contained in the extract of gambir has a similar mechanism of action as an antibacterial such as the antibiotic chloramphenicol. So that gambier extract can be used as an alternative in the treatment of typhoid fever . However, gambier extract cannot be used as a bactericidal antibacterial because it does not have the power to kill *S. typhi*.

## 4. Conclusions

Gambir extract has antibacterial activity against *S. typhi* with a MIC value of 0.5g/ml.

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