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Antibacterial activity of extract sangkareho leaves (*Callicarpa longifolia LAM.*) on *Salmonella typhi* and *Staphylococcus epidermidis*

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ABSTRACT

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Background: The most commonly found disease-causing microorganisms are the *Salmonella typhi* and *Staphylococcus epidermidis* bacteria. *S.typhi* is a gram-negative bacterium that causes typhoid fever, and *S.epidermidis* is a gram-positive bacterium that attacks mucous membranes and human skin. Sangkareho (*Callicarpa longifolia Lam.*) is one of the indigenous herbal plants of Central Kalimantan, which is empirically used as a wound medicine, diarrhea, diabetic, anti-inflammatory, and can be an antibacterial agent.

Objective: This study used six groups are concentrations of 10%, 25%, 50%, and 100%, Chloramphenicol as a positive control, and dimethyl sulfoxide (DMSO) as a negative control. The method used for this test is the well diffusion method. In Nutrient Agar (NA) media, holes were made to insert 40 µl of each extract concentration, positive and negative control with four repetitions, then incubated for 18-24 hours at 37°C. Data analysis using the One Way Anova test.

Results: The research findings showed that ethanol extract of sangkareho leaves with all concentrations had an inhibitory effect against both bacteria tested.

Conclusion: The ethanol extract of sangkareho leaves at the concentration of 10% was found to be the optimum and at the concentration of 100% was the maximum effectiveness for inhibiting *S. typhi* and *S. epidermidis*.

Latar Belakang: *Mikrooganisme penyebab penyakit yang umum ditemukan pada manusia adalah bakteri Salmonella typhi dan Staphylococcus epidermidis. S.typhi merupakan bakteri gram negatif yang menyebabkan demam tifoid dan S.epidermidis adalah bakteri gram positif yang menyerang selaput lendir dan kulit manusia. Daun sangkareho (Callicarpa longifolia Lam.) merupakan salah satu tanaman herbal khas Kalimantan Tengah yang secara empiris digunakan sebagai obat luka, diare, diabetes, antiinflamasi dan dapat menjadi kandidat antibakteri.*

Tujuan: *Tujuan dari penelitian ini untuk mengetahui konsentrasi ekstrak etanol daun sangkareho yang mampu menghambat pertumbuhan S. typhi dan S. epidermidis*

Metode: *Penelitian ini menggunakan 6 kelompok yaitu konsentrasi 10%, 25%, 50%, dan 100%, Kloramfenikol sebagai kontrol positif dan dimetil sulfoxide (DMSO) sebagai kontrol negatif. Metode yang digunakan untuk pengujian ini dengan metode difusi sumuran. Pada media Nutrient Agar (NA) dibuat lubang untuk memasukkan sebanyak 40 µl masing-masing konsentrasi ekstrak, kontrol positif dan negatif*

dengan 4 kali pengulangan, kemudian diinkubasi selama 18-24 jam di suhu 37ºC. Analisis data menggunakan uji One Way Anova.
Hasil: *Ekstrak etanol daun sangkareho pada berbagai konsentrasi mampu menghambat pertumbuhan bakteri S. typhi dan S. epidermidis*
Kesimpulan: *Konsentrasi ekstrak etanol daun sangkareho 10% menghasilkan zona hambat optimum dan konsentrasi 100% adalah konsentrasi maksimum yang efektif untuk menghambat S. typhi dan S. epidermidis.*

INTRODUCTION

Infectious disease is one of the common health problems that can be found not only in Indonesia but also found on the other side of the world. Infectious disease is the main cause of high morbidity and mortality rate in developing countries like Indonesia. Bacteria are one of many causative agents behind infectious diseases.[1] *Salmonella typhi* and *Staphylococcus epidermidis* are common infectious microorganisms that can be found in humans.

S. typhi is a rod-shaped gram-negative bacterium that causing typhoid or commonly known as typhoid fever or enteric fever.[2] S. epidermidis is a gram-positive bacterium with mucous membrane and human skin as its targets of infection. The infection and resistance of pathogenic bacteria is the world's most concern, considering of high mortality rate in the human population.[3]

The usage of antibiotics without the right direction of use can cause an increasing phenomenon of antibiotics resistance and its side effects which worsen the patient's health state.[4] Prevention using an herbal ingredient is an alternative that can be used considering the recommendation of the World Health Organization (WHO) about the usage of herbal medicine for maintaining health, also as the prevention and treatment of chronic disease, degenerative disease, and cancer.[5]

Central Borneo is one of many provinces in Indonesia which has varieties of plants. One of the varieties that can be used as traditional medicine by the citizen is sangkareho.[6] The leaf of sangkareho (*Callicarpa longifolia Lam*.) has been empirically believed as a medicine to treat external wounds and diarrhea.[7] Sangkareho leaves contained some active substances like flavonoid, alkaloid, saponin, and steroids. Based on research performed by Eko Kusumawati et al, it is proven that the ethanol extract of sangkareho leaves has inhibitory power against the growth of *Escherichia coli* with the concentration of 25% and inhibit the growth of *Staphylococcus aureus* with the concentration of 10%.[8] Based on that, the aim of this study was to develop the potential of plant-based medicine, which is traditional medicines that functioned as antibacterial and effectiveness of ethanol extract of sangkareho (*Callicarpa longifolia Lam*.) leaves using Ultrasound-Assisted Extraction (UAE) extraction method for inhibiting the growth of *S.typhi* and *S.epidermidis* that haven't been researched before. The other objective of this research was to determine the high concentration to inhibit the growth of *S.typhi* and *S.epidermidis* using agar well diffusion method.

METHODS

Tools and materials

Materials are 5000 gr sangkareho (*Callicarpa longifolia Lam*.) leaves taken from Muara Teweh, Central Borneo, 30 µg of Chloramphenicol, dimethyl sulfoxide (DMSO), Nutrient Agar (NA), Mc. Farland 0.5, NaCl (0,9%), ethanol 70%, aquadest, *S.typhi* ATCC 78 and *S.epidermidis* ATCC 12228. Tools required are pH meter, centrifuge, analytical balance, 60 mesh strainer, stirring rod, filter paper, black cloth, reaction tube, funnel, incubator, LAF (Laminar Air Flow), Erlenmeyer flask, autoclave, rotatory evaporator, water bath, medical sterilizer, freezer, inoculation loop, and vernier caliper.

Extraction methods and phytochemical test

This research was approved by the Ethics Committee for Medical Research, Faculty of Medicine, University of Palangka Raya (No.11/UN24.9/LL/2020). 5000 grams sangkareho leaves are washed, then dried for 3-7 days,

Figure 1. Leaves, stems and roots of Sangkareho (Callicarpa longifolia Lam.)

and to make simplicia using blender and then sifted using 60 mesh strainer. The extract was made using the UAE method, in which 150 gr sangkareho simplicia for and 750 ml of ethanol 70% was added, then put the mixture to rest for 15 minutes (ratio for sample and solvent are 1:5 g/ml).[9] The next step was to put the mixture in an ultrasonic cleaning bath for 30 minutes. The extraction result was then filtered using filtering paper and evaporated in a rotary vacuum evaporator with 100 mbar pressure, in 71ºC temperature, 100 rpm. After the extract was measured, to identify substance consisted in sangkareho leaves used quantitative phytochemical analysis for flavonoids, tannins, terpenoids, saponins, and alkaloids

Antibacterial activity

The sample was taken with randomization, which the colony of S.typhi and S.epidermidis that grow in a media. Sample size using a completely randomized which is repetition 4 times in each treatment extract of 10%, 25%, 50%, and 100% with positive control (Chloramphenicol) and negative control (Dimethyl Sulfoxide/DMSO).

Sangkareho leaves inhibit the growth of S.typhi and S.epidermidis were determined by well diffusion method. The first step taken was suspending 11,2 grams of Nutrient Agar (NA) to 400 ml sterilized aquadest, then boiled until heated and stirred using a magnetic stirrer, sterilized in 121ºC autoclave for 15 minutes. Bacteria samples were put in Nutrient Agar (NA) media and incubated for 24 hours at 37ºC. Broth turbidity was adjusted to 0.5 McFarland (1.5×108 CFU). Then each bacterium (from two isolates) was inoculated by streaking the swab over the entire sterile agar surface.

30 ml NA poured and was set still until it solidifies. A sterilized cotton stick was put into the suspension, then strained on the side of the test tube, and the cotton stick is scratched on the surface containing NA until it spread evenly using a triangle rod stirrer. Let it sit still until the bacterial suspension was absorbed into the media. Then a hole is made on agar using a perforator. Into that hole was poured 40 µl sangkareho leaves extract in many concentrations, positive control (Chloramphenicol) and negative control (DMSO) was following their repetitions. A petri dish was incubated for 24 hours at a temperature of 37ºC. Antibacterial effects will be shown from the clear zone formed around the hole by measuring the inhibition zone diameter (IZD) to the nearest mm using a vernier caliper. Statistical analysis against sample using One-Way ANOVA test and continued using Tukey method.

RESULTS

Extraction result

The yield using UAE methods obtained from 1566 grams of sangkareho leaves simplicia was 8.3%.

Phytochemical screening

Active compound content in sangkareho leaves (*Callicarpa longifolia Lam*.) was shown the content of active substances in Table 1. The highest contents of active compounds from the extract of sangkareho leaves are terpenoids and flavonoids.

Table 1. Sangkareho leaves (*Callicarpa longifolia Lam*.) extract phytochemical screening result

Parameter	Method	Contents	Description
Flavonoids (mg EQ/gram)	Spectrophotometry	80.333 ± 4.646	Triplo
Tannins (mg/mL GAE)	Spectrophotometry	1.768 ± 0.006	Triplo
Terpenoids (mg/mL GAE)	Spectrophotometry	91.800 ± 3.000	Triplo
Saponins (%)	Gravimetric	12.464 ± 0.121	Triplo
Alkaloids (%)	Gravimetric	35.263 ± 0.559	Triplo

Antibacterial activity

Antibacterial activity towards S.typhi and S.epidermidis were determined by well diffusion method with 4 groups sangkareho leaves extract concentrations 10%, 25%, 50%, and 100% also positive control using Chloramphenicol and negative control using DMSO explained in table 2 and the graph shown in Figure 2 and 3.

Table 2. Average results of inhibitory zone using well diffusion method of sangkareho leaves (*Callicarpa longifolia Lam*.) extract against *S. typhi* and *S. epidermidis*

Antibacterial activity	Concentration	Inhibitory zone diameter (mm)		Inhibitory response
		Mean	SEM	
Salmonella typhi	10 % Extract	12.675 ± 0.6647*	0.2973	Strong
	25 % Extract	14.9 ± 0.4583*	0.2049	Strong
	50 % Extract	16.725 ± 0.8043*	0.3597	Strong
	100% Extract	21.25 ± 0.9341*	0.4177	Very strong
	Chloramphenicol	29.2 ± 0.1581*	0.0707	Very strong
	DMSO	0 ± 0	0	
Staphylococcus epidermidis	10 % Extract	12.95 ± 0.76032*	0.34002	Strong
	25 % Extract	15.68 ± 0.50695*	0.22672	Strong
	50 % Extract	20.54 ± 0.66933*	0.29933	Strong
	100% Extract	24.52 ± 0.73959*	0.33076	Very strong
	Chloramphenicol	30.12 ± 0.63008*	0.28178	Very strong
	DMSO	0 ± 0	0	

Notes: Superscript with (*) shown significant differences ($P<0.05$) ± is a data deviation standard from 4 repetitions. DS= Deviation Standard; SEM = Standar error of the mean.

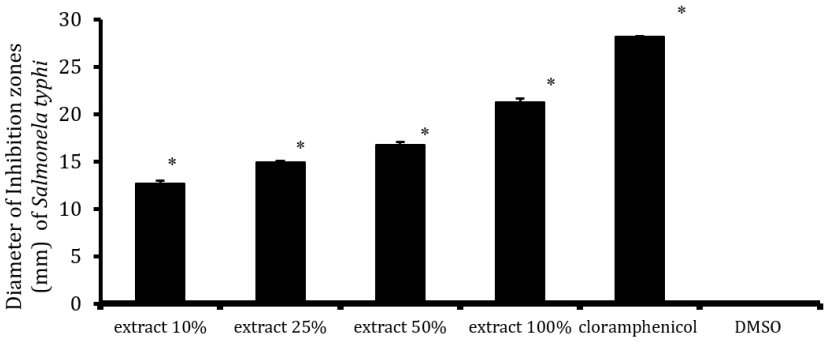

Figure 2. Average diameter of inhibition zones of *S.typhi* that formed in NA media from treatment and control groups. Note: sign asterisk (*) shown significant differences ($p<0.05$).

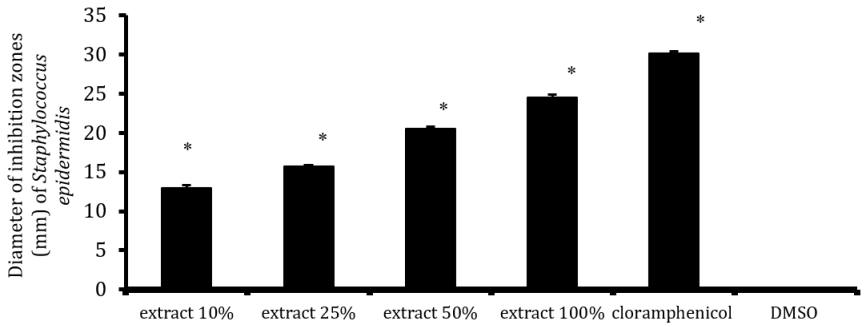

Figure 3. The average diameter of inhibition zones of *S.epidermidis* that formed in NA media from treatment and control groups. Note: sign asterisk (*) shown significant differences ($p<0.05$).

DISCUSSION

The result of phytochemical screening were carried out by obtaining secondary metabolite compounds in sangkareho leaves (*Callicarpa longifolia Lam*.), including saponins, alkaloids, flavonoids, tannins, and terpenoids. Akhmad (2016) shown that sangkareho leaves (*Callicarpa longifolia Lam*.) contained alkaloids, flavonoids, and steroids.[10] Various secondary metabolites contained in sangkareho leaves (*Callicarpa longifolia Lam*.) had an antibacterial activity with different mechanisms of action and worked synergistically.[11]

The highest contents of active compounds from the extract of sangkareho leaves, based on the phytochemical test, are terpenoids, flavonoids, and alkaloids. The mechanism of action of terpenoids as an antibacterial is by interfering with the process of forming bacterial cell membranes, forming strong polymer bonds and damaging porins, and reducing the permeability of bacterial cell walls.[2] Flavonoids have three mechanisms of action as an antibacterial which are by inhibiting nucleic acid synthesis, inhibiting cell membrane function, and energy metabolism. Flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of intercalation between flavonoids and bacterial DNA.[12] Alkaloids can interfere with the components of peptidoglycan constituent compounds in bacterial cells so that the cell wall layer is not fully formed and causes death to the bacterial cells. Extraction process using UAE method had many advantages than other conventional maceration methods, was efficiency with large yields with relatively short to operating time. Extraction method using UAE has increase the yields 1.02-2.66% because ultrasonic waves make swelling and hydration occurs so that pores of cell wall wider and heat made diffusion

increased.[13]

In this study, the antibacterial activity test of the ethanol extract of sangkareho leaves (*Callicarpa longifolia Lam.*) used the concentration of 10%, 25%, 50%, and 100%. In addition, this study also used the antibiotic Chloramphenicol as a positive control and DMSO 40 µl as a negative control, as well as an extract diluent to match the required concentration. The results of the antibacterial activity test showed that DMSO, which acted as a negative control, did not provide an inhibition zone. This proves that the NA media used in this study was not contaminated by other bacteria. DMSO does not affect antibacterial activity because it does not contain bioactive compounds that inhibit bacterial growth, so there was no inhibition zone formed. Positive control of Chloramphenicol provides an inhibition zone with an average diameter of 29-30 mm, which means that the antibiotic is sensitive in inhibiting the growth of *S.typhi.* and *S.epidermidis* because the diameter of the inhibition zone formed was > 21 mm based on the CLSI (Clinical and Laboratory Standard Institute) standard table. Chloramphenicol is a broad-spectrum antibiotic that is sensitive to gram-positive and gram-negative bacteria.[14]

There was a large increase in the diameter of the bland zone as the concentration of the extract increased. This is because the higher the concentration used, the higher the concentration of antibacterial compound metabolites contained in it. The action of terpenoids, flavonoids, and alkaloids which are quite a lot contained in the extract of sangkareho leaves, is effective as an antibacterial which can be seen from the strong inhibition zone against *S.typhi* and *S.epidermidis*. According to the category of inhibition zone formed, the extracts of the sangkareho leaves with concentrations of 10%, 25%, and 50% had a strong inhibition zone, and 100% concentration was very strong against *S.typhi* and *S.epidermidis*. The higher the concentration of antimicrobial substances, the more effective the inhibition zone produced and the greater its ability to control and kill microorganisms.[15,16] According to research conducted by Rastina et al. (2015) in Trisia et al. (2018) an effective concentration is the concentration whose antibacterial power is categorized as strong and which is closest to the concentration of the diameter of the inhibition zone formed by the positive control.[17] Inhibitory response of sangkareho leaves extract at a low concentration of 10% was considered strong for *S.typhi* and *S.epidermidis*. The maximum inhibitory response was effective at 100% concentration of sangkareho leaves extract because it was very strong and close to the concentration of the Chloramphenicol inhibition zone diameter. The large increase in the inhibition zone was caused by several factors, which are the concentration of the extract, the content of antibacterial compounds, and the nature of the bacterial cell wall itself. The more concentrated the concentration of an extract, the more secondary metabolites contained in it will affect the diameter of the inhibition zone formed.[17,18]

Diameter inhibition zone of *S.epidermidis* bacteria was larger than *S.typhi* bacteria because *S.epidermidis* (gram-positive) has thicker peptidoglycan than *S.typhi* (gram-negative). The ethanol extract of sangkareho leaves which is polar can easily penetrate the polar peptidoglycan in the cell wall of *S. epidermidis*, while the *S. typhi* bacteria contain a lot of non-polar lipopolysaccharides so that it is difficult for the ethanol extract of sangkareho leaves to penetrate.[19] Hence, a greater antibacterial effectively was shown in gram-positive than gram-negative.

CONCLUSION

Ethanol extract of sangkareho leaves (*Callicarpa longifolia Lam*.) has antibacterial activity that can inhibit the growth of *S.typhi* and *S.epidermidis*, shown from inhibition zone made up. The smallest concentration of ethanol extract from sangkareho leaves (*Callicarpa longifolia Lam*.) that can inhibit the growth of *S.typhi* and *S.epidermidis* is in 10% concentration, and the most effective and optimum is in 100% concentration.

CONFLICT OF INTEREST

All authors declared that there was no conflict of interest in this study.

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