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Effect of Extraction Method on Antimicrobial Activity Against Staphylococcus Aureus of Tapak Liman (Elephantopus Scaber L.) Leaves

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Abstract---*This study aims to compare the antibacterial activity of Staphylococcus aureus from tapak liman leaf extract using different extraction methods, maceration, and soxhletation. Maceration is a type of cold extraction, without using temperature. Meanwhile, soxhletation is a type of extraction that involves temperature in the process. The solvent used in this research is ethanol 96%. It is known that 96% of ethanol has a good safety level. The results of the phytochemical screening of tapak liman leaf extract from the two methods showed no difference in phytochemical content. The phytochemical content of tapak liman leaf extract from the two extraction methods are alkaloids, flavonoids, phenolics, and tannins. To determine the antibacterial activity in this study, the ZOI method using discs was used. The tool used to determine the inhibition ability of tapak liman leaf extract was a ruler with an accuracy level of 0.5 mm. Based on the results of the antibacterial activity test, it is known that the tapak liman leaf extract using the sokhletation method provides better inhibitory ability than the tapak liman leaf extract using the maceration method.*
Keywords---*elephantopus scaber l, extraction, staphylococcus aureus, tapak liman, temperature.*

Introduction

Indonesia is one of the countries with the largest biodiversity, which has more than 30,000 species of high-level plants. Until now, 7000 plant species have been recorded for their known properties, but less than 300 plants are used as raw materials for the pharmaceutical industry regularly. It was recorded by WHO in 2008 that 68% of people still used traditional medicine using herbs. Also, it is known that 80% of people still use herbs to treat health (Saiffudin *et al.*, 2011; Zonyfar *et al.*, 2019)

Tapak liman (*Elephantopus scaber* L.) is one of the widely spread plants in Indonesia. Mentioned previous research that Tapak Liman was used by locals of North Sulawesi (Minahasa, Mongondouw, and Sangihe tribes) for kidney, hepar, and snake bites remedies. Leaves of the plant were boiled for use (Rumouw, 2018). Another research stated that Tapak Liman extracted by 70% ethanol could reduce blood uric acid levels (Azter, 2009). The ethanol extract of Tapak Liman leaves is also known for its analgetic effect on male white mice, given orally 100, 300, and 900 mg/kg BB (Dharma, 2013). Tapak Liman was discovered to have active chemical compounds, flavonoids, alkaloids, and steroids. The study mentioned that the method used was ethanol extraction (Nonci *et al.*, 2014; Horwitz *et al.*, 1992; Smedsgaard, 1997). One of the methods used for the discovery of traditional medicine is by using the extraction method. The choice of extraction method depends on the properties of the material and

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compound to be isolated. Before choosing a method, the extraction target needs to be determined first (Sarker *et al.*, 2006).

Phytochemical screening is an initial stage to identify the content of a compound in simplicia or plants to be tested. Phytochemistry or plant chemistry studies a wide variety of organic compounds formed and stored by plants, namely regarding their chemical structure, biosynthesis, scientific distribution, and their biological function. Chemical compounds as a result of secondary metabolites have been widely used as dyes, poisons, food aromas, medicines, and so on and there are many types of plants used by medicines known as traditional medicines so that research is needed on the use of nutritious and nutritious plants. know the chemical compounds that function as medicine. Chemical compounds that are the result of secondary metabolism in plants are very diverse and can be classified into several groups of natural compounds, namely saponins, steroids, tannins, flavonoids, and alkaloids (Puspadewi et al., 2013). Several previous researchers commonly used ethanol for extracting Tapak Liman leaves (Azter, 2009; Dharma *et al.*, 2013; Nonci *et al.*, 2014). Therefore, this study will compare different extraction methods to figure out an antimicrobial activity against *Staphylococcus aureus* bacteria.

Methods

Tools and materials used in research

The tools used in this research are shaker for shaking the extract, Erlenmeyer, rotary evaporator, beaker glass, porcelain cup, water bath, measuring cup 10 ml, 50 ml, 100 ml, filter papper, spatula, oven, autoclave, biosafety, plate, cakram. The materials used in this research are tapak liman leaves simplicia, ethanol 96%, H_2SO_4 2N, mayer, wagner, and dragendrof reagen, HCl, aquadest, FeCl, *S, aureus.*

Soxhletation

25 grams of Tapak liman leaves Simplicia wrapped in filter paper, then added into the lead. 250 ml of ethanol 96% was used as the extraction solvent. The extraction was done in several cycles and stopped after the solvent that enters the lead was colorless. The extraction product then was evaporated to yield the desired extract.

Maceration

25 grams of Tapak Liman leaves were put into a maceration bottle and filled with an appropriate solvent. The chosen solvent should dissolve all secondary metabolites contained. 96% of ethanol was used to extract for 24 hours, using a 1:10 ratio between Tapak Liman and ethanol. The maceration type used was kinetic maceration. The generated macerate was then evaporated using vacuum distillation, condensed with a rotary evaporator until thick extract was obtained. The yield was weighed and documented.

Yield calculation

It is calculated using the following formula (Wika *et al.*, 2019):

$$\% \text{ yield} = \frac{\text{total weight of extract in the form of paste (g)}}{\text{total weight of simplicia (g)}} \text{ x } 100\%$$

Phytochemical screening test

Phytochemistry is an initial analysis method to examine the content of chemical compounds in plants. The expected result could provide information with specific pharmacological effects and incite more new drug discoveries. Qualitative method to test active compounds was not done on all parts of the plant, but on certain parts which were used by people as remedies ingredients (Rumouw, 2018).

1) Alkaloids
   4 grams of medicinal plant extract was added with chloroform sufficiently, the solution was then filtered into a test tube, 10 drops of H2SO4 2N was added to the filtrate. The mixture was shaken routinely, left for a few minutes to form 2 layers. The top layer was transferred into 3 test tubes, 1 ml each. A few drops of Mayer,

Wagner, and Dragendorf reagent were added to each tube, then a white, brown, and orange precipitate was formed respectively.

2) Flavonoids
   200 mg of medicial plants in the form of fine powder was extracted using 5 ml of ethanol and heated in a test tube for 5 minutes. Then a few drops of concentrated HCl were added. After that, 0.2 grams of Mg powder was added. A positive result was indicated by the dark red color appearance for 3 minutes.
3) Saponins
   2 grams of medicial plants in the form of fine powder was put into a test tube and added with distilled water until soaked. The test tube was boiled for 2-3 minutes and then shaken after cooled. A positive result was indicated by the formation of a stable foam.
4) Phenolics
   Before started to identify phenolic compounds, the sample was continuously extracted using a soxhlet device with ether as a solvent to dissolve the fat and chlorophyll. After ether extraction, then continue with 50% methanol to bind polar components, 1 ml of ethanol extract plus 5% FeCl. A color change from brownish yellow to orange-brown indicated the presence of phenolic compounds.
5) Tannins
   20 mg of medicial plants in the form of fine powder was added with ethanol until completely soaked. Then 2-3 drops of 1% FeCl were added. The formation of a bluish-black or green color indicated the presence of tannin compounds.

An antimicrobial activity using Zone of Inhibition (ZOI)

The stage is carried out to carry out the zone test the inhibition is by preparing the agar culture that has been inoculated with *S.aureus* bacteria lawn pattern on the entire surface of the medium in order, tapak liman extract using different methods. After it was created the pit using a good number with a diameter of ± 6 mm. Every hole is filled 30 µl with different sample concentration variation 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0,39% with using a micropipette.

Subsequently incubated in an incubator on temperature 37 ° C 18-24 hours. To find out the diameter of the zone of inhibition that is by looking at the clear zone around the well. Inhibition zone measurement using a ruler to an accuracy of 0.5 mm (Tortora *et al.*, 2016).

Results

Yield Calculation

Table 1
Comparison of yield value of Tapak Liman extract using soxhletation and maceration

Extract methods	% yield
Maceration	13,40%
Soxhletation	15,88%

Based on the data above, it is known that the yield weight of the extract using the soxhletation method is greater than the maceration method.

Phytochemical screening

Table 2
Phytochemical screening of Tapak Liman extract using soxhletation and maceration

Phytochemicals	Maceration	Soxhletation
Flavonoids	+	+
Tannins	+	+
Phenolics	+	+
Alkaloids	+	+
Steroid terpenoids	+	+
Saponins	-	-

Based on the data above, there was no content difference between the two extraction methods used.

Antimicrobial activity test results

Table 3
Antibacterial activity test results of Tapak Liman extract using soxhletation and maceration

Concentration (PPM)	Inhibition Zone	
	Soxhletation	Maceration
100.00	10,23 mm ± 0,25	9,17 mm ± 1,62
50.00	9,45 mm ± 0,69	3,65 mm ± 5,16
25.00	7,33 mm ± 2,19	5,13 mm ± 4,47
12.50	4,33 mm ± 3,79	0,00 mm ± 0,00
6.25	3,50 mm ± 4,95	2,40 mm ± 4,16
3.13	6,50 mm ± 0,71	2,40 mm ± 4,16
1.56	0,00 mm ± 0,00	0,00 mm ± 0,00
0.78	3,00 mm ± 4,24	2,07 mm ± 3,58
0.39	0,00 mm ± 0,00	0,00 mm ± 0,00

Discussion

Maceration is a cold extraction method and the simplest, in which the solvent will penetrate the plant's cell wall and enter the cell cavity containing the active substance so that the actives which is the concentrated solution will be pushed out of the cell due to concentration difference between the solutions outside and inside the cell. (Wahyulianingsih *et al.*, 2016).

Soxhletation is a hot-cold method. In this extraction process, the solvent and sample are placed separately. In principle, the extraction is continuously done using a relatively minimum solvent. When the extraction is complete, the solvent will be evaporated and an extract is obtained. Commonly used solvents are the volatile ones or have low boiling points (Leba, 2017).

Ethanol has properties that can dissolve all actives contained in natural ingredients, polar, semipolar, and also nonpolar actives. Also, ethanol was found to be easier to penetrate cell membranes to extract intracellular elements from plants (Tiwari *et al.*, 2011). Ethanol also has a low enough boiling point that it can be easily evaporated without uses a high temperature, is inert, and has a great price affordable (Rohmaniyah, 2016). Ethanol polarity is 5.2 which means the solvent tends to be more universal, so it can attract metabolite compounds secondary which are polar, semipolar, and nonpolar (Adham *et al*., 2019; Tsiodras *et al.*, 2001; Fukai *et al*., 2002; Hur *et al.*, 2004).

Based on the results, it is known that soxhletation resulted in higher yield. This is also supported by research conducted by Puspitasari & Prayoga (2017), which shows that the yield produced by extraction with the Soxhletation method is greater than the maceration method. The advantage of the soxhletation method is the continuous extraction process and the sample is extracted by pure solvent resulting from condensation so that the yield produced is more than the method maceration extraction. Setyowati *et al.* (2014), claimed that this has happened because higher extraction temperature will cause faster molecular movement, as well as the solvent circulation (migration). The temperature and solvent circulation factors can increase the transfer rate of compound mass from leaf cells, thus intensify the contact frequency of the solute and solvent, and more extract is obtained.

One of the extract quality parameters is the yield of the extract produced. The yield is the ratio between the extract obtained and the initial simplicity. The yield uses percent (%) units, the higher the yield value produced indicates the value of the extract produced is more (Armando, 2009). The yield of an extract can be influenced by several factors, one of which is the extraction method used (Ministry of Health of the Republic of Indonesia, 2000).

Based on research conducted by Puspitasari & Prayoga (2017), it is known that the yield value of the extract from the Soxhletation method is greater than the maceration method. followed by determining the phenolic content of the two extraction methods, it was known that the phenolic content of the cherry leaves studied was greater in the extract used by the Soxhletation method compared to the maceration method. This is also supported by research conducted by Desmiaty *et al.* (2019), which shows that extraction using the Soxhletation method has a greater value of polyphenols and antioxidants.

Based on the phytochemical screening test results, there was no difference in content found between the two extraction methods. This is because the same solvent was used so that it does not affect identifiable content. Identifiable phytochemicals in Tapak Liman extract were flavonoids, tannins, alkaloids, phenolics, and terpenoids.

Alkaloids can disrupt peptidoglycan constituent in bacterial cells, causing the call wall failed to form completely and ultimately causing cell death (Taufiq, 2015). Tannins also have antibacterial activity by denaturing bacterial cell proteins, causing inhibition in bacterial cells (Ramadani, 2013). The mechanism of action of phenol as an antimicrobial is by denaturing cell proteins (Pelczar *et al.*, 2010). Terpenoids' mechanism as an antimicrobial is to react with porin (transmembrane protein) in the outer membrane of the bacterial cell wall, forming a strong polymer bond, resulting in porin destruction (Owan *et al.*, 1999 in Budifaka, 2014).

While flavonoids' mechanism as an antimicrobial is divided into 3, inhibiting nucleic acid synthesis, inhibiting cell membrane functions, and inhibiting energy metabolism (Hendra *et al.*, 2011). Flavonoids may link to soluble proteins located outside the cells and with bacteria cell walls thus promoting the formation of complexes flavonoids also may act through inhibiting both energy metabolism and DNA synthesis thus affecting protein and RNA syntheses (Bouarab-Chibane *et al.*, 2019). Based on the antimicrobial activity test results, it was found that at the concentration, antimicrobial activity is higher in the soxhletation method. These findings are aligned with the explanations above.

Conclusion

It is known that the soxhletation method produces greater yield, and there is no difference in phytochemical screening between the two methods. while the results of the S. aureus antibacterial activity test were greater using the Soxhlet method.

Suggestion

Further research is needed to determine the content of each extraction method.

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