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Phytochemical Profile and Antibacterial Activity Test of Medicine Plants in Southeast Sulawesi Against Salmonella Typhi YCTC as An Alternative Addition to Substitution Ingredients for Halal Products

Muhammad Jefriyanto Budikafa[1]*; Sahidin[2]; Rini Hamsidi[3]
[1]UIN Prof. K.H. Saifuddin Zuhri Purwokerto, Indonesia
[2]Universitas Halu Oleo Kendari, Indonesia
[3]Universitas Airlangga Surabaya, Indonesia
Correspondence email: muh.jefriyantob@uinsaizu.ac.id

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Abstract
Phytochemical profile and antibacterial activity test performed on several medicinal plants methanol extracts of plants Keji beling (Strobilanthes crispus BI), Starfruit (Averrhoa bilimbi Linn.), Kangkung (Ipomoea aquatica), Secang (Caesalpinia sappan Linn.) and Jathropa (Jatropha curcas Linn.) existing in the Southeast Sulawesi. Phytochemical screening carried out by TLC method to identify classes of secondary metabolic compounds like alkaloids, flavonoids, tannins, saponins and triterpenoids, while the antibacterial activity test against the bacteria Salmonella typhi YCTC using the agar diffusion method with chloramphenicol as a positive control. The results obtained show that the extract of the roots and leaves of plants of S. crispus yield a diameter of 9 mm and 5 mm clear zone, the extract of the leaves of plants A. bilimbi yield a diameter of 9 mm clear zone, while the extract of the trunks and flowers of C. sappan. plants produce a clear zone diameter of 3,5 mm and 6 mm against the bacteria. extracts from plant parts that have activity against salmonella bacteria can then be further investigated to obtain compounds that have potential as antibacterial and can be used as alternatives as herbal medicinal ingredients in halal products.

Keywords: phytochemical; antibacterial; salmonella; typhi yctc; alternative ingredients; halal product.

INTRODUCTION

Infectious diseases always occupy the top list of causes of morbidity and mortality in developing countries, including Indonesia. The infection that occurs is influenced by the causative microorganism that enters, the degree of virulence and the body's immunity so that people with infections usually take drugs that contain antibiotics (Wahyono, 2012). Most of the infectious diseases that are harmful to humans are caused by microorganisms, one of which is the bacterium Salmonella typhi which causes an acute infectious disease, namely typhoid fever. (Pelczar dan Chan, 2007).

Typhoid fever in people with low standards of living and hygiene tends to increase and is endemic. Sources of transmission of typhoid fever are active patients, patients in the convalescent phase and chronic carriers. Typhoid fever is also known by other names, namely Typhus abdominalis, Typhoid fever or Enteric fever. Typhoid fever is an acute systemic illness characterized by fever, headache and abdominal discomfort lasting for at least 3 weeks accompanied by abdominal symptoms, enlargement of the spleen and skin eruptions. (Supriyono, 2011).

Antibacterials that are given immediately with a clinical diagnosis of typhoid fever are the first-line antibacterial groups. Based on the efficacy and price, currently chloramphenicol is still the first choice, but the drawback is that long-term administration can cause resistance. The factors that cause this resistance are the use of antibacterial without a prescription or without a doctor's guidance and control, choice of antibacterial, dosage, and inappropriate duration of antibacterial administration. (Kemenkes, 2006).

Research on substances with antibacterial properties needs to be carried out to find new antibacterial compounds that have the potential to inhibit or kill bacteria that are resistant to antibiotics at an affordable price. One alternative that can be taken is to utilize the active substances contained in plants (Khunaifi, 2011).

Medicinal plants have long been the target of the search for new drugs. The development of the use of drugs, especially from plants, to help improve public health status is quite widespread. One of the benefits of using drugs from these plants in humans is as an antibacterial (Awoyinka et al., 2007).

The usefulness of medicinal plants in general is actually caused by the chemical content they have. However, not all chemical constituents are completely known because chemical examination of a plant is expensive (Hariana, 2006).

Southeast Sulawesi consists of various ethnic groups, and each tribe has many families. This will make an important contribution to ethnobotanical diversity (Ruslin dan Sahidin, 2008). This diversity also makes a difference in how to use plants for the treatment of diseases, including in the treatment of infectious diseases where there are still some people who use empirical knowledge in the use of plants as antibacterial drugs, some of which have been known and commonly used by the community for generations as efficacious plants. as a medicine such as the plant Keji Beling (Strobilanthes crispus BI), Starfruit (Averrhoa bilimbi Linn.), Water spinach (Ipomoea aquatica), Secang (Caesalpinia sappan Linn.) and Jatropha (Jatropha curcas Linn.). Therefore, the community needs to be given information about plants that have the potential as antibacterial so that people's knowledge about traditional medicinal plants will increase which will increase the welfare of the community.

Based on the above background, it is necessary to carry out further experimental research to explore the antibacterial potential of the plants Keji Beling (Strobilanthes crispus BI.), Starfruit (Averrhoa bilimbi), Kangkung (Ipomoea aquatica), Secang (Caesalpinia sappan L.) and Jatropha (Jatropha curcas) as an antibacterial against the growth of S. typhi bacteria for

an easy and safe alternative therapy for the community as well as providing information about the content of existing secondary metabolites through phytochemical screening of plants.

This study aims to determine the classes of compounds and the antibacterial activity of plant parts of Keji Beling (S. crispus), Starfruit (A. bilimbi), Kangkung (I. aquatica), Secang (C. sappan) and Jatropha (J. curcas)). This study used bacteria from the Yogyakarta Culture Type Collection culture, namely S. typhi YCTC. The antibacterial activity test was carried out using the agar diffusion method, by comparing the activity of the test substance with a reference antibiotic (chloramphenicol). extracts from plant parts that have activity against salmonella bacteria can then be further investigated to obtain compounds that have potential as antibacterial and can be used as alternatives as herbal medicinal ingredients in halal products.

LITERATURE REVIEW

There have been several previous studies on the topics discussed, including (Zega et al., 2021), (Megawati, 2020), (Ernilasari et al, 2021), (Widowati et al, 2021), and (Dubale et al, 2023). Broadly speaking, previous research contains similarities and differences with this research. This can be described as follows.

Research conducted by Zega et al (2021) entitled "Antibacterial activity test of Simargaolgaol (Aglaonema modestumSchott ex Engl) leaves extract against Escherichia coliand Salmonella typhi bacteria" aims to to determine the antibacterial activity of n-hexane, ethyl acetate and ethanol extracts from Simargaolgaol leaves against Escherichia coliand Salmonella typhi bacteria.. The results of antibacterial activity showed thatthe highest inhibitory power of Simargaolgaolleaf extract was ethanol extract (polar),ethyl acetate extract (semi polar) and n-hexane extract (non polar). The inhibitory power of ethanol extract, ethyl acetate, and n-hexane against Escherichia colibacteria was 13.1 mm (strong); 9.7 mm (medium); 8.0 mm (medium) and for Salmonella typhibacteria respectively 11.2 mm (strong); 10.7 mm (strong) and 9.3 mm (medium). With the concentration of the extract in the diameter is 10%. Based on this, it can be concluded that Simargaolgaolleaf extract has potential as an antibacterial.

Research conducted by Megawati (2020) entitled " Phytochemical Screening, Secondary Metabolites and Biological Activities of Southeast Sulawesi Plants" The results of research on phytochemical screening and isolation of secondary metabolites from local researchers indicated that the plants from Southeast Sulawesi have the potential to be studied further. Plants from Southeast Sulawesi show pharmacological potential activities that can be developed for the purpose of treating diseases caused by bacteria, fungi, cancer, and as an antioxidants. There are Southeast Sulawesi endemic plants from the Etlingera genus that are interesting to explore (phytochemical screening, isolation of pure compounds and pharmacological studies) considering that of the 14 species of Etlingera plants scattered in Southeast Sulawesi, 2 (two) of which have recently been reported, namely E. elatior and E. calophrys.

Ernilasari (2018) research entitled "Antibacterial activity of leaves, flowers, and fruits extract of Etlingera elatiorfrom Nagan Raya District, Indonesia against Escherichia coliand Staphylococcus aureus," which aims to o determine the best concentration of ethanol extract of leaves, flowers, and fruits of E. elatioras an inhibitor against Escherichia coli and Staphylococcus aureus. The results showed that the fruit extract of E. elatior has antibacterial activity with an effective inhibitory zone at a concentration of 2% is 8.4 mm (E. coli) and 2.4 mm (S. aureus). Meanwhile, antibacterial activity of the extract of leaves and flowers against E. elatiorcannot determine yet.Identification of leaves, flowers and fruits extract of E. elatiorusing GC-MS (gas chromatography-mass spectroscopy) showed 56 compounds were detected.

The study entitled " Phytochemical Screening and Antibacterial Activities of Senggani (Melastoma malabathricumL.) Ethanolic Extract Leaves" aims to to perform phytochemical screening and to know the pathogenic antibacterial activities of senggani leaves extract. The results showed that ethanolic extract of senggani leaves has eight active compounds, those are phenolics, alkaloids, flavonoids, tannins, triterpenoids, glycosides, steroids, and saponins. This further proved that the ethanolic extract of senggani leaves have antibacterial activity and are able to inhibit the growth of all bacteria tested. The best ability shown to inhibit E. colibacteria was at concentration of 100%, Sh. dysenteriaeand P. aeruginosastarted at the concentration of 75%, and S. aureusstarted at the concentration of 50% (Widowati et al, 2021).

Based on the results of this study, there are similarities with this study in that several medicinal plants have antibacterial activity against salmonella typhi, so that in this study several plants will be tested which are known to have medicinal properties against salmonella typhi bacteria, in addition to that, a phytochemical profile of each plant will be tested to see the metabolite content. secondary from each of these plant extracts.

RESEARCH METHOD

The methods used in this research are:

1. Sample Collection and Preparation

The samples in this study were the Keji shard (S. crispus), Starfruit (A. bilimbi) and Kangkung (I. aquatica) plants obtained from the gardens of the residents of Monapa Village, Mowila District, South Konawe Regency while the Secang plants (C. sappan) and Jatropha (C. sappan) J. curcas) was obtained in the area around Kendari City. Sample preparation was carried out by cleaning the sample first and then drying it in aerated manner before cutting it into small pieces and then blending it into a powder. All of the powder is then weighed 50 - 100 grams from each part of the plant and stored in a plastic container (plastic bottle) for further processing.

2. Maceration Extraction

Maceration extraction of plant powders was carried out in a closed container for 3 x 24 hours using methanol as a solvent. Separation of residues and filtrates was carried out every 1 x 24 hours accompanied by the replacement of the same solvent. The maserate is separated from the dregs by filtering using a funnel and filter paper and then evaporated with a Rotary Vacuum Evaporator at 56°C to obtain a thick extract. The extract was then weighed and obtained different weights.

3. Preparation of extract solution with a concentration of 10,000 ppm

The concentration of the 10,000 ppm extract used in this method was determined through a preliminary test with reference to the previous method. The concentration was made by dissolving 0.5 gram of extract in 50 ml of methanol to obtain an extract with a concentration of 10,000 ppm.

4. Phytochemical Screening

Phytochemical tests with TLC were carried out using silica gel GF_{254} plates. Extract from each plant was spotted at a distance of ± 1 cm from the bottom edge of the plate with a capillary tube then dried and eluted with chloroform-methanol (9:1) mobile phase. After the mobile phase movement reached the boundary line, the elution was stopped. The stains on the surface of the plate were examined under UV light at a wavelength of 254 nm, 366 nm and visible light, then given a testing reagent for each class of compounds. The test reagents for each class of compounds are as follows:

a. The group of alkaloid compounds used Dragendorff reagent to detect those showing orange brown spots (Lutfillah, 2008).
b. The flavonoid group is steamed with ammonia vapor to produce a greenish yellow color (Kusnaeni, 2008).
c. The tannin compound group was used as a 1% $FeCl_3$ sprayer to produce a light blue color (blackish blue). (Harborne, 2003).
d. The saponin group when added to H_2SO_4 causes a dark purple-purple color (Kristianingsih, 2005).
e. The triterpenoid compound used the Lieberman-Burchard reagent to produce a red-purple (violet) color.

5. Antimicrobial activity test

a. Sterilization of tools and materials

Petri dishes, Erlenmeyer, test tubes, spatulas, filter paper, Nutrient Agar (NA), and all tools and materials (except methanol and plant extracts) to be used are sterilized in an autoclave at 121°C and 15 Psi pressure for 15 – 20 minutes after previously being washed, dried, and wrapped in paper (Pelczar dan Chan, 2005).

b. Media preparation

Media for bacterial growth used nutrient agar (NA). The media was prepared by weighing 20 grams of NA media, dissolved in 1,000 ml of distilled water in an Erlenmeyer and heated using a hot plate until it boiled and completely dissolved and then sterilized in an autoclave at 121°C for 15 minutes.

c. Bacterial culture preparation

The test bacteria used in this study were S. typhi YCTC bacteria which came from Prof. Sahidin. The bacteria used are rejuvenated by transferring 1 or 2 ose grown on NA media, then put in a test tube and incubated for 24 hours at 37 ± 2°C

d. Preparation of bacterial suspension

Standard 0.5Mc Farland suspension

A total of 0.05 mL of $BaCl_2$ was mixed with 9.95 mL of 1% H_2SO_4 in a test tube after which it was homogenized, where the 0.5 Mc Farland suspension was a standard suspension which showed bacterial turbidity equal to 108 CFU/mL.

The way it is made is that the bacteria to be tested are suspended by growing the bacteria in sterile NaCl liquid media. Bacterial turbidity was measured according to Mc standard. Farland 0.5 using 20D spectronics at λ 625 nm.

e. Antibacterial activity testing

Antibacterial activity was tested by agar diffusion method using disc paper. This method is carried out by mixing 1 ml of each bacterial suspension into 15 ml of agar media which has been diluted in a sterile petri dish and then allowed to solidify. Disc paper with a diameter of 6 mm which has been dripped with 20 µl of each extract (10,000 ppm) (Adyana dkk., 2004), control solvent (methanol) and standard antibiotics (chloramphenicol) were added to the surface of the media and then incubated at 37°C for 1 x 24 hours. The results of the antibacterial activity test were based on measuring the diameter of the inhibition area (DDH) of bacterial growth formed around the disc paper.

RESULTS AND DISCUSSION

1. Sample Preparation

Sample preparation begins with wet sorting with running water which aims to remove impurities present in the sample. then dried before being cut into small pieces. The aim of cutting the sample is to facilitate the processing into powder, then a dry sorting process is carried out to separate impurities, foreign organic matter and simplicia that are damaged due to the previous process. The sample is then blended into a powder where the smaller the size of a particle will increase the surface area of the sample so that the interaction between the sample and the solvent

will be maximized. All of the powder is then weighed 50 - 100 grams from each part of the plant and stored in a plastic container (plastic bottle) for further processing.

In the manufacture of extracts from each plant the selected extraction method is the maceration method. This method was chosen because it has several advantages, including the absence of a heating process so that the unstable compounds are not damaged or lost by the presence of heat, the way to work is easy, the equipment is simple and easy to work on (Anonymous, 1986). Maceration was carried out in a closed container for 3 x 24 hours using methanol as a solvent. Separation of residues and filtrates was carried out every 1 x 24 hours accompanied by the replacement of the same solvent. The purpose of soaking for 24 hours is to precipitate unwanted compounds that are also extracted in the solvent. The maserate is separated from the dregs by filtering using a funnel and filter paper and then evaporated with a Rotary Vacuum Evaporator at 56°C to obtain a thick extract.

2. Phytochemical Profile Test

The phytochemical profile test in this study was carried out using the TLC method. The TLC method is used because it is simple, inexpensive, and can analyze several components simultaneously. Tests were carried out to determine the content of secondary metabolites contained in each plant methanol extract. The secondary metabolites tested in this study were alkaloids, flavonoids, tannins, saponins and triterpenes.

The eluent used as the mobile phase in this study was chloroform-methanol (9:1), the same eluent was used for all classes of compounds because the focus of this study was to identify which classes of secondary metabolites were present in each plant extract.

The results of the alkaloid compound class test were obtained based on the visible stains on the TLC plate after each extract was spotted on the plate and eluted with the mobile phase and then added Dragendorff reagent as a stain sighter. Extracts containing alkaloid compounds will produce an orange brown color which is a reaction between bismuth and mercury nitrate with alkaloid compounds as shown in Figure 1.

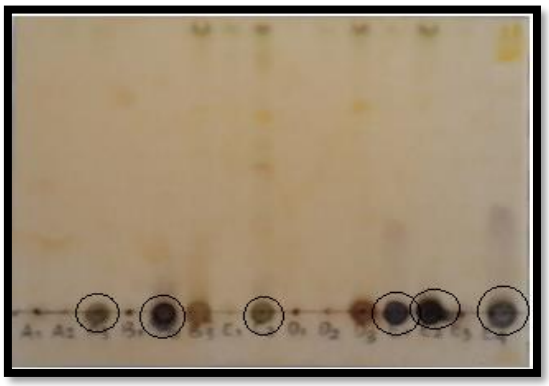

Figure 1. Alkaloid Compound Group Test

The results of the test for the class of flavonoid compounds were obtained based on the stains that appeared on the TLC plate after each extract was spotted on the plate and eluted with the

mobile phase and then steamed with ammonia vapor. Positive test results will show a greenish yellow color as shown in Figure 2.

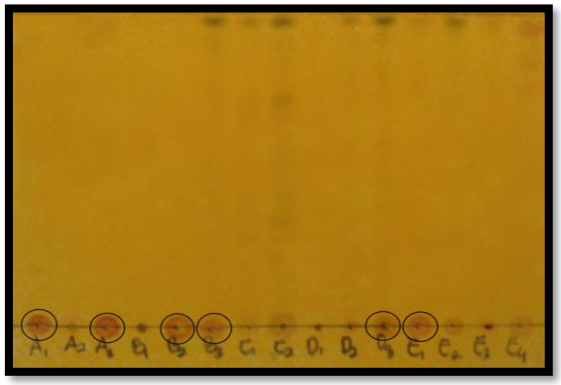

Figure 2. Flavonoid Compound Group Test

The results of the tannin compound class test were obtained based on the stains that appeared on the TLC plate after each extract was spotted on the plate and eluted with the mobile phase and then 1% $FeCl_3$ was added as a stain sighter. A positive test result will produce a violet (blue-black) color as shown in Figure 3.

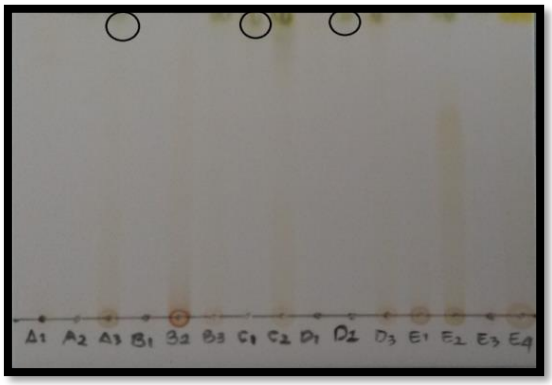

Figure 3. Tannins Compound Group Test

The test results for the saponin compound class were obtained based on the stains that appeared on the TLC plate after each extract was spotted on the plate and eluted with the mobile phase and then added H_2SO_4. A positive test result will produce a dark purple-purple color as shown in Figure 4.

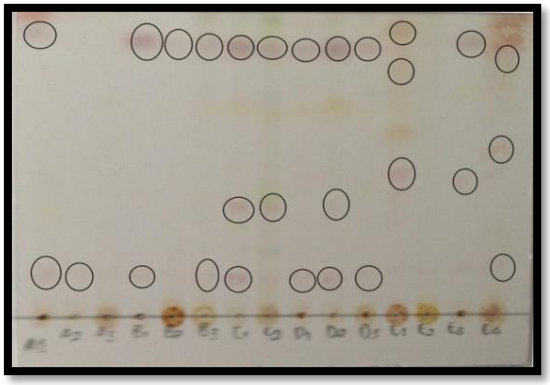

Figure 4. Saponin Compound Group Test

The results of the triterpene compound test were obtained based on the stains that appeared on the TLC plate after each extract was spotted on the plate and eluted with the mobile phase and then Lieberman-Burchard reagent was added. Positive test results will produce a red-purple (violet) color as shown in Figure 5.

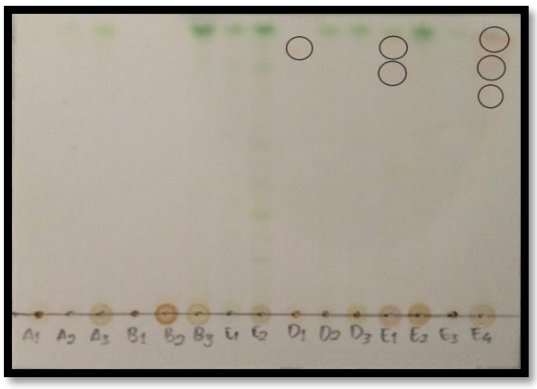

Figure 5. Triperpene Compound Group Test

3. Antibacterial Activity Test

Antibacterial activity test in this study was carried out on S. typhi YCTC bacteria. This test was conducted to determine the inhibition of methanol extract from each plant extract and chloramphenicol against S. typhi YCTC bacteria. The samples used in this study were Keji Beling (S. crispus), Starfruit (A. bilimbi), Kangkung (I. aquatica), Secang (C. sappan), Jatropha (J. Curcas). Each extract was diluted with a concentration of 10,000 ppm and then tested on the test bacteria to see the activity of the plant in inhibiting the growth of the tested bacteria.

The results of measuring the diameter of the inhibition zone on the growth of S. typhi YCTC were obtained from the measurement of the diameter of the clear zone formed around the disc paper, then the value was adjusted to the inhibition zone diameter indicator, with the interpretation that if a value of < 3 mm was obtained on the clear zone diameter measurement, it means the inhibition response weak, 3-6 mm means moderate resistance response and if it is > 6 mm the response is strong resistance (Pan et al, 2009).

Table 1. Antibacterial activity test results

Sample		**DDH (mm)**	**Interpretation**
Keji Beling (*Strobilanthes crispus*)	Root	**9**	Strong
	Stem	**0**	No activity
	Leaf	**5**	Medium
Starfruit (*Averrhoa bilimbi*)	Root	**0**	No activity
	Stem	**0**	No activity
	Leaf	**9**	Strong
	Stem	**0**	No activity

Sample		DDH (mm)	Interpretation
Kangkung (*Ipomoea aquatica*)	Leaf	**0**	No activity
Jathropa (*Jatropha curcas*)	Root	**0**	No activity
	Stem	**0**	No activity
	Leaf	**0**	No activity
Secang (*Caesalpinia sappan*)	Stem	**3,5**	Medium
	Leaf	**0**	No activity
	seed	**0**	No activity
	flower	**6**	Medium

The research data above shows that there are several extracts that do not show inhibitory activity as an antibacterial against S. typhi YCTC while the results of the phytochemical profile contain secondary metabolites that may be active as an antibacterial. There are several factors that can influence this. Each compound has a different configuration so that the biological effect on each compound is also different even though it is in the same group of metabolites and the large number of active compounds in the extract makes it easier for the active compounds to damage bacterial cells, no inhibition zones are formed in some extracts due to low active compounds that can cause inhibition of the growth of the tested bacteria (occurs in a small proportion of the total number of bacterial cells) so that bacteria that are not disturbed by low levels of active compounds can continue to grow.

Alkaloids, flavonoids, tannins, saponins, and triterpenes are compounds that have the same structure as the positive control used, namely chloramphenicol where these compounds have an aromatic ring which is also present in chloramphenicol so that it can be said that the aromatic ring has antibacterial activity. therefore further research is needed to ascertain the activity of each class of compounds related to the structure they have.

Chloramphenicol as the positive control is a compound with the molecular formula $C_{11}H_{12}Cl_2N_2O_5$ which is used as an antibiotic that is bacteriostatic and has a broad spectrum. Chloramphenicol acts by inhibiting protein synthesis by binding to the 30 S subunit on the bacterial cell ribosome and inhibiting the activity of the peptidyl transferase enzyme quickly without interfering with DNA and RNA synthesis. (Marjono, 1995).

The mechanism of action of alkaloids as an antibacterial is through inhibition of cell wall synthesis which will cause cell lysis so that the cell will die. Alkaloid compounds have a base group containing nitrogen which will react with amino acid compounds that make up the bacterial cell wall and bacterial DNA. This reaction results in changes in the structure and arrangement of amino acids, which will cause changes in the genetic balance of the DNA chain resulting in damage and encourage lysis of bacterial cells which will cause cell death in bacteria (Tanaka et al., 2006).

The mechanism of the class of flavonoid compounds as antibacterial through their ability to form complexes with extracellular proteins and soluble proteins and bacterial cell walls (Dwidjoseputro, 2010). The complex bonding of flavonoid compounds with bacterial cell proteins through hydrogen bonds makes the bacterial cells unstable because the bacterial cell protein structure becomes damaged due to hydrogen bonds with flavonoids, so that the bacterial cell proteins lose their biological activity, as a result the permeability function of the bacterial cells is disrupted and the bacterial cells will experience lysis which results in the death of bacterial cells (Harbone, 2003).

The mechanism of action of tannins can shrink the cell wall or bacterial cell membrane so that it can interfere with cell permeability. As a result of disturbed permeability, bacterial cells cannot carry out living activities so that their growth is stunted or even dies (Ajizah, 2004 in Rahmadani, 2013).

The mechanism of action of the saponin compound class will change the surface tension and bind to lipids in bacterial cells which causes lipids to be excreted from the cell wall so that the permeability of the bacterial membrane is disrupted (Wardhani and Nanik, 2012).

The mechanism of terpenoids as an antibacterial is reacting with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymer bonds that result in damage to the porin. Damage to the porin which is the entry and exit gate for compounds will reduce the permeability of the bacterial cell wall which will result in a lack of nutrients in the bacterial cell so that the growth of the bacteria is inhibited or dies (Cowan, 1999).

In this case, one group of active compounds which functions as an antibacterial cannot be determined with certainty. In order to determine with certainty which class of compounds is active as an antibacterial, it is necessary to carry out further examination of each of these compound groups. Apart from the group of compounds identified using thin layer chromatography (TLC), it is also possible that there are metabolites/other groups of active compounds in the methanol extract of each plant that have not been identified and have potential as antibacterial against S. typhi YCTC.

CONCLUSIONS

1. Secondary metabolites of the alkaloid compound group are found in methanol extracts of the roots and leaves of keji beling (S. crispus), the stems and leaves of starfruit (A. bilimbi), the leaves of jatropha (J. curcas) and the stems of secang (C. sappan).
2. Secondary metabolites of the flavonoid compound group are found in the methanol extract of the leaves of keji beling (S. crispus), the stems of kangkung (I. aquatica) and the stems of jatropha (J. curcas).
3. Secondary metabolites of the tannin compound group are found in the methanol extract of the leaves of keji beling (S. crispus), the stems of starfruit (A. bilimbi), the leaves of kangkung (I. aquatica) and the stems, leaves and flowers of secang (C. sappan).

4. Secondary metabolites of the saponin compound group are found in the methanol extract of the roots and stems of Keji Beling (S. crispus), all parts of starfruit (A. bilimbi), all parts of kangkung (I. aquatica), all parts of Jatropha (J . curcas) and stem parts, seeds and flowers of secang(C. sappan).
5. Secondary metabolites of the triterpene compound group are found in the methanol extract of the roots of jatropha (J. curcas) and the stems and flowers of secang(C. sappan).
6. Extract of the roots and leaves of plants of S. crispus yield a diameter of 9 mm and 5 mm clear zone, the extract of the leaves of plants A. bilimbi yield a diameter of 9 mm clear zone, while the extract of the trunks and flowers of C. sappan. plants produce a clear zone diameter of 3,5 mm and 6 mm against the bacteria. extracts from plant parts that have activity against salmonella bacteria can then be further investigated to obtain compounds that have potential as antibacterial and can be used as alternatives as herbal medicinal ingredients in halal products.

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