Phytochemical Analysis and Antibacterial Activity of Medicinal Plant Kecapi (*Sandoricum koetjape* Merr)

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Abstract: Indonesia is a mega-biodiversity country that has around ± 3,000 plant species, which are plants that produce medicinal ingredients. The traditional use of medicinal plants in Indonesia is increasingly preferred because the side effects are smaller than synthetic drugs and are very easy to obtain. One of the medicinal plants that are widely used in Central Sulawesi is the kecapi plant. Kecapi belongs to the Meliaceae family and is commonly used in traditional medicine as a medicine for digestive system disorders, heartburn medicine, ulcer medicine, eye pain medicine, fever medicine, and cough medicine. Parts of plants commonly used as traditional medicine are leaves, fruit, bark, fruit skin and roots. This study aimed to determine the content of secondary metabolic compounds and the antibacterial activity of the medicinal plant kecapi. Phytochemical analysis using the Harbone method and antibacterial activity using by using the well diffusion method. The results showed that the leaf extract of the kecapi (Sandoricum koetjape Merr.) contained flavonoids, saponins, tannins, and terpenoids. Kecapi leaf extract was very strong in inhibiting the growth of Staphylococcus aureus bacteria at concentrations of 75%, 100% and at concentrations of 25% and 50% categorized into strong categories

Keywords—*Medicinal plant, Phytochemical Analysis, Antibacterial Activity, Staphylococcus aureus*

I. INTRODUCTION

Indonesia is one of the countries with the second largest forest owner after Brazil [1], [2], with a forest area of 120.7 million ha [3]. Indonesia's tropical forests have a high diversity of plant species and the potential as a medicine source [1], [2]. About ± 3,000 species are medicinal plants [4]. One type of medicinal plant that has the potential to be developed is *Sandoricum koetjape* Merr [5]. Medicinal plants are prevalent in Indonesian society as an alternative treatment, especially for people who live around forests, and are a means of supporting public health for generations, long before formal health services and modern medicines touched the community in the village [1]. Medicinal plants are preferred because the community is cheap and easy to obtain and has fewer side effects than synthetic drugs. The high cost of synthetic medications makes people turn to medicinal plants. The use of medicinal plants by the village community includes preventing disease, maintaining body freshness, and treating disease. One of the areas in Indonesia that uses a lot of medicinal plants is Sigi Regency, Central Sulawesi. Sigi Regency is a district where most of the area includes forest areas, so many of its residents live around the forest and use medicinal plants to treat various diseases. One of the medicinal plants used is the kecapi plant.

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medicinal plants is Sigi Regency, Central Sulawesi [8]. Sigi Regency is a district where most of the area includes forest areas, so many of its residents live around the forest and use medicinal plants to treat various diseases. One of the medicinal plants used is the kecapi plant [7].

Kecapi is a native plant from Malaysia, Cambodia, and Southern Laos. It has long been known by people in Indonesia, the Philippines, India, and the Andaman Islands [9]. Kecapi belongs to the Family of Meliaceae and is widely used in traditional medicine as a medicine for digestive system disorders, heartburn medicine, ulcer medicine, eye pain medicine, fever medicine, and cough medicine, as well as infectious diseases in humans. Parts of plants that are commonly used as traditional medicine are leaves, fruit, bark, fruit peel, and roots [10]

Pathogenic bacteria are one of the causes of infectious diseases in humans [11]. The type of pathogenic bacteria that causes many contagious diseases is Staphylococcus aureus [12]. Infection is a disease that often occurs because microorganisms enter the body, causing disturbances in the body's normal physiology. Staphylococcus aureus is a bacterium that causes infections in humans that infect the skin [13]. The skin protects the inside of the body from physical or mechanical disturbances, heat or cold disturbances, interference with radiation or ultraviolet rays, and interference with germs, bacteria, fungi, or viruses [12].

Currently, the use of the leaves of the kecapi plant for the treatment of various diseases caused by staphylococcus bacteria by the people in Sigi Regency is only based on hereditary practice and has not been proven empirically. Another reason for the use of this plant as a medicine is to reduce the level of resistance to the use of antibiotics. To empirically prove the local knowledge of the community about the medicinal plant kecapi, it is necessary to research the secondary metabolic content and anti-bacterial activity of Staphylococcus aureus in the ethanol extract of the kecapi plant.

II. MATERIAL AND RESEARCH METHOD

A. Research Site and preparation of plant extracts

Kecapi leaf samples were taken from the Sigi District Forest Area, Central Sulawesi, Indonesia. Phytochemical analysis was carried out at the Research Laboratory, Department of Chemistry, and the antibacterial activity test was carried out at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University, Indonesia. The leaves of the medicinal plant kecapi are washed with water. After cleaning, the kecapi leaves are dried in direct sunlight to dry. The dried leaves are then mashed using a blender, and the powder is sifted. The kecapi leaves that have become powder are then extracted by maceration. The sample was soaked with 96% ethanol solvent in a ratio of 1:10 for 24 hours with stirring. The maceration results were then filtered with Whatman 42 filter paper to produce filtrate and residue. Immersion was carried out three times until the filtrate was close to clear. The filtrate was then concentrated with a vacuum rotary evaporator at a temperature of 40oC to obtain a crude extract as a paste.

B. Phytochemical analysis

Phytochemical analysis was a test for alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and carotenoids [14].

Flavonoid Test

A total of 2 ml of the kecapi leaf sample solution was put into a test tube, and then added Mg powder, and a few drops of concentrated HCl (Shinoda reagent) was. A positive result was indicated by a change in colour to orange, pink or red [1], [14].

Alkaloid Test

2 ml of kecapi leaf extract was put into a test tube, and 2-3 drops of Dragendorf reagent were added. Positive results are indicated by forming an orange precipitate [1], [14].

Saponin Test

A total of 2 ml of the kecapi leaf sample solution was put into a test tube, added with distilled water, and shaken for a few minutes. The formation of foam or froth indicates positive results for 15 minutes [1], [14].

Tannin Test

A total of 2 ml of kecapi leaf sample solution was put into a test tube, and a few drops of 5% FeCl3 were added. Positive results are indicated by the appearance of brownish-green or blue-black [1], [14].

Terpenoid Test

A total of 2 ml of the extract was mixed with 0.5 ml of chloroform, then 1.5 ml was concentrated to form a layer, and then H2SO4 was added. Positive results are indicated by the formation of reddish-brown color on the surface [1], [14].

Steroid Test

A total of 2 ml of the extract was dissolved in 5 ml of chloroform, and then 6 ml of concentrated sulfuric acid was added to the side of the tube. Positive results are indicated by forming a red top layer and a lower layer of sulfuric acid showing yellow and green colors [1], [14].

Carotenoid Test

A total of 2 ml of the extract was mixed with 5 ml of chloroform in a test tube and then shaken, filtered, and added with 1 ml of acetic anhydride. Positive results are indicated by the formation of blue color on the surface [1], [14].

C. Staphylococcus aureus antibacterial activity test

The kecapi leaf extract was tested for its antibacterial activity against Staphylococcus aureus using the diffusion well method [8]. The bacterial suspension was made by inoculating one needle loop of pure culture of Staphylococcus aureus from stock culture into 15 ml of Nutrien Broth medium. Then it was incubated at 37ºC for 24 hours until the test bacteria density was equivalent to 10-6 CFU. First, a sterile Petri dish was filled with 80 ml of bacterial culture suspension. 15 ml of Nutrient Agar (NA) media was poured and then shaken simultaneously so that the bacteria could grow evenly. Then, let it stand until the media solidifies; after the media has solidified, a diffusion

well with a diameter of 5 mm is made. Then add 80 ml of extract, then incubate at 37ºC for 24 hours. Next, measure the diameter of the clear zone formed.

D. Data Analysis

The data obtained from this study were analyzed using analysis of variance in a completely randomized design model with four treatments and three replications; if there is a significant difference in each treatment, it can be continued using the LSD (Least Significant Difference) test [15].

III. RESULTS AND DISCUSSION

A. Phytochemical analysis of kecapi (Sandoricum koetjape Merr) leaf extract

The phytochemical analysis of the kecapi leaf extract showed that the positive extract contained flavonoids, saponins, tannins, and terpenoids. However, no alkaloids, carotenoids, and steroids were detected in total, which is presented in table 1.

Table 1. Phytochemical Analysis of Kecapi (*Sandoricum koetjape* Merr) Leaf Extract

Compounds	Test Results	Description
Flavonoids	+	Changes in colour to orange
Alkaloids	-	No orange precipitate formed
Tannins	+	Appearance of brownish green or black blue
Saponins	+	Formation of foam or froth
Steroids	-	There is no color change and no layer is formed on top of the solution
Terpenoids	+	Formation of reddish brown color on the surface
Carotenoids	-	No color change

Phytochemical analysis was conducted to determine the secondary metabolite compounds contained in the kecapi leaf extract [16]. The phytochemical analysis is classified as qualitative because it can only identify the active compounds contained in the kecapi leaves without knowing the levels of these active compounds [17].

Flavonoid Test

Flavonoid compounds are natural phenolic compounds and are polar compounds because they have some unreplaced hydroxyl groups or sugar so that they will dissolve in polar solvents such as methanol, ethanol, acetone, butanol, and water [1]. The results of the phytochemical test showed that the extract of the kecapi leaves contained positive. Flavonoid compounds have anti-bacterial and tumour properties [18]. Suitable reducing compounds that inhibit many oxidation reactions are flavonoids, both enzymatically and non-enzymatically; an antioxidant that plays a role in inhibiting cancer cells is a flavonoid [19]. In the plant world, flavonoids include many of the most common pigments ranging from fungi to angiosperms. The ability of flavonoids to stop the early stages of the reaction, therefore flavonoids, can suppress tissue damage by free radicals, inhibit lipid peroxidation and inhibit several enzymes [20]. Flavonoid compounds are beneficial in inhibiting bleeding and antibiotics [18].

Alkaloid Test

The results of the alkaloid test, as shown in table 1, showed that the kecapi leaf extract was negative for alkaloid compounds. Alkaloid compounds have health benefits, including triggering the nervous system, fighting microbial infections and lowering or increasing blood pressure [21].

Saponin Test

The results of the saponin test on the kecapi leaf extract were positive for saponin compounds. Saponin compounds have been used for generations by local communities in rural areas around the world as anti-bacterial and anti-cancer compounds and have other pharmacological properties [1], [22], [23]. Saponins have polar properties so that they can be dissolved in solvents such as water [24]. Compounds that are soluble in nonpolar (hydrophobic) solvents as surfactants can reduce surface tension and foam that arises because saponins contain compounds that are partially soluble in water (hydrophilic) [23], [24].

Tannin Test

The results of the tannin test on the kecapi leaf extract were positive for tannins, as shown in Table 1. The research results on tannin compounds from various plant extracts have anti-bacterial and anti-bacterial properties [8], [22]. This is following local wisdom showing that this type of kecapi leaf is used as an anti-bacterial.

Terpenoid Test

The results of the terpenoid test on kecapi leaf extract were positive for terpenoid compounds, as shown in Table 1. The results of research about kecapi leaves also contain triterpenoid chemical compounds [25]. The phytochemical test of terpenoids was carried out by adding chloroform and H_2SO4. Terpenoid compounds will form ions that give several colour reactions and will be dehydrated by adding the solid acid H_2SO4. The occurrence of an oxidation reaction in the terpenoid group is due to a colour change through the formation of a conjugate double bond [26]. Triterpenoid compounds show significant pharmacological activities, such as anti-viral, anti-bacterial, and anti-inflammatory, as inhibiting cholesterol synthesis and as anti-cancer [27].

Steroid Test

The steroid tests on kecapi leaf extract were negative for steroid compounds, as shown in Table 1. *Steroid compounds* are essential in the pharmaceutical field and are one of the compounds widely used in medicine, such as anti-inflammatory, pain-relieving and anti-bacterial drugs.

Carotenoid Test

The results of the carotenoid test carried out on the negative kecapi leaf extract contained steroid compounds, as shown in Table 1. The same study was) on the phytochemical and bioactivity screening of the root extract of *Uncaria nervosa* Elmer (Bajakah), using methanol as solvent where the phytochemical test results on the bark and root bark extracts showed that both the bark and root bark extracts contained secondary metabolites of alkaloids, flavonoids, terpenoids, and phenolics, while saponins and quinones were absent in either the bark or root bark extracts [28]. Carotenoids have benefits as antioxidants that can protect the body from free radicals [29].

B. Inhibitory test of kecapi leaf extract against Staphylococcus aureus

The Eboni leaf extract activity test was carried out using the well diffusion method with six treatments, namely extract concentrations of 25%, 50%, 75% and 100%, and positive control of 2% Chloramphenicol and negative control of distilled water. Further test results from each treatment on the formation of inhibition zones on the growth of Staphylococcus aureus can be seen in the following table 2:

Table 2. Antibacterial activity of Staphylococcus aureus lute leaf extract.

Treatment	Mean	LSD 5%
P1 = Control (-)	0.0 a	
P2 = 25%	17.3 b	
P3 = 50%	17.7 b	**3.38**
P4 = 75%	21.3 c	
P5 = 100 %	21.5 c	
P6 = control (+)	35.2 d	

The results of the antibacterial activity test of kecapi leaf extract can inhibit the growth of Staphylococcus aureus bacteria which is characterized by the presence of an inhibition zone around the wells/holes made for each treatment. The zone of inhibition did not occur in the negative control treatment or distilled water, proving that the solvent used did not inhibit bacterial growth. The inhibition zone of kecapi leaf extract can be grouped into three categories, namely very strong, forming a clear zone of 20 mm. Strong inhibition zone forming a clear zone of 11-20 mm. Moderate inhibition forms a clear zone of 6-10 mm, and weak inhibiting has an area of a clear zone of 5 mm [8], [30]. Based on these categories, the kecapi leaf extract at concentrations of 70% and 100% were categorized as very strong. Concentrations of 25% and 50% were categorized as strongly inhibiting the growth of *S. aureus* bacteria.

The test results in table 2 show that increasing the concentration of kecapi leaf extract will also increase the diameter of the resulting inhibition zone. The highest inhibition zone of kecapi leaf extract was obtained when the extract was given with a concentration of 100%, but the inhibitory power produced was smaller than the inhibition obtained from the positive control (Chloramphenicol 2%).

The solid inhibitory power of kecapi leaf extract is due to the content of secondary metabolites contained in the extract. The results of the phytochemical analysis showed that this extract contained flavonoid compounds, tannins, saponins and terpenoids. Saponins and terpenoids have been proven to be antibacterial compounds [31]. The mechanism of action of saponins is included in the antibacterial group, which interferes with the permeability of bacterial cell membranes, which results in cell membrane damage and causes the release of various essential components from the bacterial cell, namely proteins, nucleic acids and nucleotides [32]. The cytoplasmic membrane works to maintain certain materials in the cell and regulate the flow of other materials in and out. The cytoplasmic membrane also provides the biochemical equipment for moving mineral ions, sugars, amino acids, electrons, and other metabolites across the membrane. Damage to the membrane will result in the inhibition of cell growth or cell death [8], [32], [33].

Other secondary metabolic compounds found in kecapi leaf extract are terpenoids. One of the compounds derived from terpenoids that have antibacterial properties is triterpenoids; this compound is a group of compounds derived from natural ingredients that are widely distributed [34]. The triterpenoid compounds found in kecapi extract have been shown to have significant pharmacological activities, such as anti-viral, antibacterial, and anti-inflammatory, as inhibition of cholesterol synthesis and as anti-cancer [35].

The ability of kecapi leaf extract activity in inhibit the growth of Staphylococcus aureus bacteria is also classified based on the National Clinical Laboratory Standards (NCCLS) in forming a clear zone which can be grouped into three categories, namely the inhibition zone is classified as a sensitive category, if the diameter formed is more than or equal to 20 mm. Resistance category if the diameter formed is less than or equal to 10 mm. Intermediate category if the diameter formed is between 11-19 mm. [36]. Based on this, it can be seen that the inhibitory ability of kecapi leaf extract is categorized as sensitive because it has an inhibitory zone diameter of 20 mm at concentrations of 75%, 100% and 2% chloramphenicol. In comparison, 25% and 50% concentrations were categorized into the intermediate category because they had an inhibition zone diameter of 11-19 mm.

IV. CONCLUSION

The local knowledge of the people of Sigi Regency about the medicinal plant kecapi is empirically proven that the plant can be used as a medicine to prevent infection in humans. The phytochemical analysis results showed that the kecapi leaf extract (Sandoricum koetjape Merr.) was positive for flavonoid compounds, saponins, tannins, and terpenoids. The kecapi leaf extract was very strong in inhibiting the growth of Staphylococcus aureus bacteria at concentrations of 75% and 100% and concentrations of 25% and 50% categorized into strong categories.

"Temperature (K)", not "Temperature/K".

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