Jurnal Penelitian dan Kajian Ilmiah Kesehatan
Politeknik Medica Farma Husada Mataram
Volume 10 Issue 1 2024
DOI : 10.33651/jpkik.v10i1.692
Website : https://jurnal.poltekmfh.ac.id/index.php/JPKIK

ANTIBACTERIAL ACTIVITY OF JAPANESE CAMBOJA (*Adenium obesum*) LEAF EXTRACT ON THE GROWTH OF THE BACTERI *Sthaphylococcus aureus*

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ARTICLE INFO

Article History:
Received
Accepted
Published online

Keywords: Japanese frangipani leaf, *Staphylococcus aureus*

ABSTRACT

Staphylococcus aureus bacteria are microorganisms that can causing the most common infections such as skin infections. In the treatment of infections commonly use antibiotics, but there are often problems in use of antibiotics, leading to resistance. One way Preventing resistance is by looking for alternatives that are better cheap and efficient, one example is by using frangipani plants. (*Adenium obesum*). Japanese frangipani leaves themselves are believed to contain alkaloids, flavonoids and saponins that function as antibacterials. The purpose of this study was to determine the effect of giving Japanese frangipani leaf extract (*Adenium obesum*) on the growth of *Staphylococcus aureus* bacteria. This research method is a laboratory experiment by means of wells diffusion with concentrations of 50%, 25%, 15% and 10%. The results of this study indicate that there is no clear zone in each concentration. The conclusion is that Japanese frangipani leaves (*Adenium obesum*) are unable to inhibit the growth of *Staphylococcus aureus* bacteria.

INTRODUCTION

Bacteria are microorganisms that can cause infection and is still a health problem in the world (Purnamasari, Vifta and Susilo, 2018). *Staphylococcus aureus* is a pathogenic bacterium often found in the upper respiratory tract, mouth, urinary tract, nose and skin. Based on statistical data, skin infections are among the most common diseases, especially in developing countries, one of which is Indonesia, because it is a country with a tropical climate. Skin infection (pyoderma) in Indonesia alone has a prevalence of 1.4% for adults and 0.2% for children (Suerni *et al.,* 2013). Antibiotics are usually used for treatment, as they stop or kill the progression of the infection. However, problems that are often found in the unwise use of antibiotics can cause bacterial resistance to antibiotic (Savitri, Triatmoko and Nugraha., 2020). *Staphylococcus aureus* is one of the bacteria that has a very high sensitivity and resistance to several antibiotics such as Methicillin (MRSA). MRSA cases continued to increase from 2015 to 2018, namely 7.69% in 2015, 5.63% in 2016, 10.81% in 2017 and 12.94% in 2018 (Atik Nuryah *et al.,* 2019). Not only that, several studies have also revealed that as many as 10% of MRSA positive cases were found in hospitals, especially in the ICU room (Satriani Syarif *et al.,* 2022).

The high case of resistance to bacteria is a global problem, so efforts are made to obtain alternative materials that can overcome infections caused by bacteria that are resistant to antibiotics such as the use of

natural antimicrobial medicinal plants, of course, which can be obtained from plants, one of which is the Japanese Cambodian plant. The Japanese frangipani plant (*Adenium obesum*) is an alternative medicine, all parts of the frangipani plant can be used in traditional medicine. The stems and leaves of frangipani contain flavonoids and alkaloids (Gunawan *et al.,* 2010). Frangipani bark is used by the community as an herbal remedy for patek (frambosia), anexternal remedy for cracked skin on the soles ofthe feet, while the boiled water is used to soak swollen feet and can also be used as an antibiotic(Khulafa, 2020). The flowers, leaves and bark of Japanese Cambodia (*Adenium obesum*) contain many bioactive compounds with anticancer, anti-inflammatory and antimicrobial activities(Lim, 2014). Based on the description above, it is necessary to conduct research on the antibacterial activity of Japanese frangipani leaf extract (*adenium obesum*) against the growth of *Staphylococcus aureus* bacteria.

MATERIAL AND METHOD

The type of research is a laboratory experiment, with post test only control group design. This research was conducted in the litbankkes laboratory of the NTB Provincial General Hospital. Research time in June 2024.

Tools and materials

The tools used in this study were sterile cotton swabs, test tubes, bunsen, petri dishes, inoculation needles, erlenmeyer, scissors, matches, analytical balance, autoclav, incubator, oven, hotplate, magnetic sterier, micropipette, test tube rack, yellow type, blender, baeker glass, aluminum foil, filter paper, funnel, rotary evapator. While the materials used in this research was japanese cambodian leaves, Muller Hiton Agar (MHA), pure culture of S. aureus, Standard 0.5 Mc Farland units, NaCl 0.9%, Ethanol 96%, sterile distilled water.

Sterilization of tool

all tools used are washed with water and cleaning liquid then wrapped using paper/ newspaper and then dried in an oven at 150℃ for 90 minutes.

Making muller hiton agar media

A total of 34 grams of MHA was mixed with 1 liter of distilled water in an erlenmeyer. Then heated on a hotplate while stirring using a magnetic stirrer until boiling. After boiling, the media was removed and put into an autoclave to be sterilized for 15 minutes at a temperature of 121℃ and a pressure of 1.5 atm. MHA media that has been sterile is then poured into petri dishes and allowed to solidify.

Extraction

approximately 50 grams of frangipani leaf powder was extracted by maceration using 200 ml of ethanol until the entire powder was submerged and stirred, then covered and incubated for 3 days. stirring was carried out approximately three times a day. then filtering is carried out so that the filtrate and residue areobtained. the resulting residue is then re- meserated with the addition of ethanol for 3 days, filtering is carried out every day. all the resulting filtrates are put together in one container. then the filtrate is concentrated with a vacuum rotary evapator with a temperature of40 ℃, until a thick extract is obtained.

Antibacterial activity test with the pitting diffusion method

Prepare Muller hiton agar (MHA) media, mark the petri dish with the name, date and microorganism tested, insert a sterile cotton swab into the culture of microorganisms, then turn the cotton part to the side of the tube so thatthe liquid does not drip from the tip of the cotton. spread microorganisms on the entire surface of the agar plate. o get even growth scratch horizontally, then rotate the slab 90◦ and make a second stroke rotate the slab 45◦ and make a third stroke. let the slab dry for 5 minutes. Then make a hole using the blue tip. 6 holes were made for concentrations of 10%, 15%, 25%, 50% and positive and negative controls. Insert the sample solution using a micropipette as much as 50ul into each well. For positive control using chloramphenicol antibiotic, negative control using aquadest. then incubate the plate at 37℃ for 24 hours.

RESULTS

Testing the antibacterial activity of frangipani leaf extract against the growth of *staphylococcus aureus* bacteria was carried out using the well diffusion method, where

the principle of this method is to measure the diameter of the clear zone around the wells containing antimicrobial substances.

Measurement of Inhibition Zone Diameter

The incubation process was carried out for 24 hours, then the diameter of the inhibition zone was observed.

Diameter measurements were made using a caliper with an accuracy of 0.01 mm. The diameter of the inhibition zone is measured from the edge (break point) to the edge (break point) of the opposite inhibition zone past the center of the plate If there is no inhibition zone around the wells, the inhibition zone value is said to be 0.00 mm (Hudzicki in Putra, 2016).

Samples of frangipani leaf extract have been made with a series of levels of 10%, 15%, 25% and 50%. The results of the observation of the inhibition zone of frangipani leaf extract against *Staphylococcus aureus* bacteria can be seen in table 1.

Treatment	Average zone of inhibition (mm)
	Staphylococcus aureus
50%	0,0
25%	0,0
15%	0,0
10%	0,0
K +	25,5
K-	0,0

Based on table 1 the antibacterial activity test ofJapanese frangipani leaf extract (*Adenium obesum*) in P1, P2, P3, P4 and negative control did not have an inhibition zone against *Staphylococcus aureus* bacteria**,** while the positive control using *chloramphenicol* antibiotics showed an average inhibition zone of 25.5 mm, this indicates that the bacteria used in this study are still sensitive to antibiotics.

DISCUSSION

The results of the research conducted in table 1 using Japanese frangipani leaf extract (*Adenium obesum*) with ethanol solvent as an inhibition test against the growth of *Staphylococcus aureus* bacteria with concentrations of 50%, 25%, 15%, and 10% did not form an inhibition zone. The inhibition zone was not formed allegedly due to the influence of temperature and drying time at the time of making simplisia, the drying process of the material is the most important activity because it can affect the quality of the resulting product (Yamin *et., al* 2017). In this study, drying was carried out using an oven because the heating temperature was evenly distributed and the air circulation was more perfect and could be completed in a short time. In general, the temperature for drying making simplisia is in the range of 30-90 ℃, the optimal temperature is not more than 60 ℃. According to Syafrida (2018). The higher the drying temperature, the lower the content of bioactive compounds in thesample. The temperature used is 60℃ for 2 days, drying with a very high oven temperature for a very long time causes the loss of bioactive compound content which results in the formation of no inhibition zone in Japanese frangipani leaf extract (*Adenium obesum*) as an inhibition test against the growth of *Staphylococcus aureus* bacteria.

Apart from the effect of temperature and length of drying, it is also suspected that it is due to differences in solvents used, in previous research conducted by Sulistyarsi.A (2018) which used white frangipani leaves with methanol solvent which has the same content as Japanese frangipani leaves (*Adenium obesum*) at a concentration of 25%, an inhibition zone of 23.66 mm was formed and declared very strong. Research conducted by (Putri.J.y. et al., 2013). which compared the use of ethanol solvent with methanol solvent in soursop leaf extract, the results showed that methanol solvent produced higher flavonoid levels than ethanol solvent. Methanol solvent has a higher dielectric constant value than methanol solvent, the higher the dielectric costanta of a solvent the more polar the solvent (Ramayani, 2021). Different types of solvents during extraction significantly affect the levels of active compounds produced.

This is corroborated by research conducted (Rusmiyati.I. et al., 2012) which uses methanol extract of young soursop leaves as an antibacterial against *Staphylococcus aureus* at a concentration of 25% with a very strong inhibition zone diameter, it can be indicated that the manufacture of extracts using methanol solvents has stronger antibacterial activity

compared to extracts using ethanol solvents. This research is the same as the results of research (Pormes.O.et.,al 2016) Using plucked spinach leaves with ethanol solvent as an inhibition test against *Staphylococcus aureus* bacteria which has the same active compound content as Japanese frangipani leaves (*Adenium obesum*) both do not form an inhibition zone at each concentration and negative control, only in the positive control which uses antibiotics.

CONCLUSION AND SUGGESTION

Based on the results of the research that has been done, it can be concluded that ethanol extract of Japanese frangipani leaves (*Adenium obesum*) does not have inhibition against the growth of *Staphylococcus aureus* bacteria. It is recommended for further researchers to pay more attention to oven temperature and the length of drying when making simplisia and pay attention to the type of solvent used in the extraction process.

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