Putu Sulistiawati Dewi, Rhizome Bangle Extract Inhibition Growth Test Against *Staphylococcus aureus* for Oral Abscess Herbal Treatment: Journal of Health and Dental Sciences.e-ISSN 2807-3126 Vol. 1 No. 3 : 225-232

RHIZOME BANGLE EXTRACT INHIBITION GROWTH TEST AGAINST *Staphylococcus aureus* FOR ORAL ABSCESSS HERBAL TREATMENT

(UJI DAYA HAMBAT EKSTRAK RIMPANG BANGLE TERHADAP PERTUMBUHAN Staphylococcus aureus SEBAGAI PERAWATAN HERBAL ABSES ORAL)

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Doi: 10.54052/jhds.v1n3.p225-232

Article History
Received: 18 /11/2021
Accepted: 31/12/2021

ABSTRACT

An abscess is a typical infection caused by *Staphylococcus aureus* bacteria. Resistance of bacteria occurs due to improper antibiotics dosage, inaccurate diagnosis, and improper bacterial causes. Rhizome Bangle contains saponins, flavonoids and essential oils, alkaloids, tannins, and glycosides that have antibacterial. The purpose of this study is to determine the inhibitory effect of rhizome Bangle extract (*Zingiber purpureum Roxb*.) on the growth of *Staphylococcus aureus* bacteria in Vitro. The study was an experimental laboratory study with group design post-test in vitro. The method used as the antibacterial test was the *Kirby Bauer* diffusion method. Concentration of rhizome Bangle extract were 8%, 16%, 32%, and negative control of ethanol and positive control of chlorhexidine 0,2%. The culture medium used Mueller Hinton agar (MHA). Data analyzed by Krusskal wallis ($p < 0.05$). The result of this study shows Rhizome Bangle extract 32% concentration inhibit *Staphylococcus aureus* bacteria growth significant with $p = 0.001$. The conclusion is that rhizome Bangle extract (*Zingiber purpureum Roxb*.) inhibits the growth of *Staphylococcus aureus* bacteria at 32% concentration.

Keywords: antibacterial; rhizome bangle; *staphylococcus aureus*

ABSTRAK

Abses merupakan infeksi khas yang disebabkan oleh bakteri Staphylococcus aureus. Resistensi suatu bakteri dapat terjadi karena pemakaian antibiotik yang tidak tepat dosis, tidak tepat diagnosis, dan tidak tepat bakteri penyebab. Rimpang bangle mengandung saponin, flavonoid, dan minyak atsiri, alkaloid, tanin, dan glikosida yang telah terbukti memiliki aktivitas sebagai antibakteri. Tujuan dari penelitian ini untuk mengetahui adanya daya hambat ekstrak Rimpang Bangle (Zingiber Purpureum roxb) terhadap pertumbuhan bakteri Staphylococcus aureus secara in vitro. Penelitian ini merupakan penelitian eksperimental laboratorium dengan post-test only design group secara in vitro. Metode yang digunakan sebagai uji antibakteri adalah metode difusi Kirby Bauer dengan konsentrasi ekstrak Rimpang Bangle (Zingiber Purpureum roxb) yang digunakan dalam penelitian ini yaitu 8%, 16%, 32% dan kontrol negative etanol serta kontrol positif chlorhexidine 0,2%. Media biakan yang digunakan adalah Mueller Hinton agar (MHA). Data dianalisis dengan Kruskall wallis (p<0,05). Hasil penelitian ini menunjukan ekstrak Rimpang Bangle (Zingiber Purpureum roxb) berpengaruh bermakna menghambat pertumbuhan bakteri Staphylococcus aureus dengan nilai p=0,001. Kesimpulan yang bisa diperoleh dari penelitian ini adalah ekstrak Rimpang Bangle (Zingiber Purpureum roxb) dapat menghambat pertumbuhan bakteri Staphylococcus aureus pada konsentrasi 32%.

***Kata kunci**: antibakteri; rimpang bangle; staphylococcus aureus*

INTRODUCTION

The appearances of normal flora as part of the body do not always bring advantage; in a particular condition. The normal flora can result in some diseases, for example, if there is subtracted change from its original habitat.[1] *Staphylococcus aureus is a* positive gram bacteria that result in yellow pigment, aerobic facultative, and it does produce neither spore nor motile.

Generally, it grows in couples or a group, with a diameter around 0,8- 1,0 µm. *Staphylococcus aureus* is a circular, grape-like form, grouping erratically.[2] *Staphylococcus aureus* is one of the normal microflora in the oral cavity that results in some oral problems, such as angular cheilitis, parotitis, *staphylococcal mucositis, denture stomatitis*, and form diseases through invasion to other tissues with the release of toxic. Necrosis, inflammation, and abscess condition are the sign of conditions resulting from these bacteria.[3] Abscess is a specific infection caused by *Staphylococcus aureus*. An abscess is a cavity containing pus surrounded by inflammation tissues forming from localized infection.[4]

The treatment of abscess in the oral cavity after the drainage process is done by absorbing antibiotics. However, due to the insufficient use of this antibiotic, resistance happens.[5] The resistance result from different dosage, incorrect diagnosis, and different causing bacteria. Resistance bacteria possess a defense method to avoid antibiotics, where it creates mutation inactive part or dependence part, forming protein transmembrane that code resistance gene to the antibiotic.[6]

The danger of resistance and high price medical treatment increase citizens' awareness to look for an alternative of antibiotic, where they use the traditional method, extraction of plants, one of them is Rimpang (Rhizome) Bangle *(Zingiber purpureum Roxb.).* The herb consists of saponin, flavonoid, atsiri oil, tannin, steroid, triterpenoid, antioxidants like vitamin C, vitamin E, carotene, and phenolic compound. Based on the research, rimpang bangle extraction have pharmacology activity acting as antibacterial, lactase, pancreas lipase inhibitor, and protecting the cell from damage caused by oxidative H2O2. Compounds inside the extract pull by solvent when the extraction happens, suspected to play role in many mentioned pharmacology activities.[7] This research aims to determine the inhibition of rhizome bangle extract with 8%, 16%, and 32% concentration into the growth of *Staphylococcus aureus* in vitro.

METHOD

This research uses several things: sterile test tube, erlenmayer tube, autoclave, incubator, microliter, tweezers, spatula, label paper, thermolyne, beaker glass, evaporator centrifuge, spectrophotometer, petri dish, slide bar, cotton bud, handscoon, and mask. A substance used is *Mueller Hinton Agar, Staphylococcus aureus* ATCC 43300*,* Brain Hearth Infusion Broth*,* Ethanol 96%, and Chlorhexidine 0,2%. This research is in vitro laboratory experimental

research using a post-test-only control group design.

Turbidity suspension of *Staphylococcus aureus* ATCC 43300 equivalents with 180 CFU/mL was taken by cotton bud. Afterward, it is sterilized by smearing it on the surface of Mueller Hinton. Rimpang bangle extract *(Zingiber purpureum Roxb)* with concentrations 2%, 4%, 8%, and positive control are added to disk blank as many as 6. A disk containing rimpang bangle extract with various concentrations and positive control was placed on Mueller Hinton Agar's surface with *Staphylococcus aureus* ATCC 43300 suspense. It is incubated in an incubator at 37^{o}C for 24 hours.

Observation is done after 24 hours of incubation. The colorless brood is the bacteria sensitivity code used as test materials, taken based on the obstacle zone diameter. The diameter is counted in millimeters (mm) using a slide bar. The diameter has categorized the power of anti-bacteria based on the following David and Stout classification; 20 mm diameter, or extreme obstacle zone; 10–20 mm means strong obstacle zone, 5-10 mm means moderate, and 2-5 mm means weak. The collected data analyzed by Krusskal wallis and post hoc with MannWhitney.

RESULT

The normality and homogeneity test of the data using the Shapiro-Wilk test and Levene's test shows an abnormally distributed and not homogeny. Afterward, the analysis on the treatment effectiveness is made by applying the Kruskal Wallis test. The analysis is displayed in Table 1.

Table 1. Result of obstacle zone diameter by using Kruskal Wallis test on *Staphylococcus aureus*

Variable among groups	N	Average Mean	P
negative Control	6	0	
positif control	6	9	
Rimpang bangle 8%	6	8	0,001
Rimpang bangle 16%	6	18	
Rimpang bangle 32%	6	23	

From Table 1, it is showed p=0,001, significant value ρ< 0,0. It shows that there is a significant difference among the groups. The Mann-Whitney U test is used to see the difference, and the result is shown in Table 2.

The difference between one and other groups can be seen from the value of sig (p). the significant difference is attained with a p-value <0,0. Data in Table 2 show that the extract of 8% and 16% rimpang bingle do not obstruct the *Staphylococcus aureus.* Meanwhile, 32% concentration can inhibit the growth of the bacteria.

Table 2. Result of obstacle zone diameter by using Mann Whitney U test on *Staphylococcus aureus*

Group	Z	Sig
Concentration 8% and 16%	0.00	1.00
Concentration 8% and 32%	-3.80[illegible]	0.00[illegible]
Concentration 8% and positive control	2.887	0.004
Concentration 8% and negative control	0.000	1.00
Concentration 16% dan 32%	-3.80[illegible]	0.00[illegible]
Concentration 16% and positive control	2.887	0,004
Concentration 16% and negative control	0,000	1,00
Concentration 32% and positive control	-3.[illegible]	0.00[illegible]
Concentration 32% and negative control	-3.80[illegible]	0.00[illegible]
Positive and negative control	-3.88[illegible]	0.004

DISCUSSION

The research shows that rimpang bangle with 8% and 16% concentration have no obstacle zone, the 32% of it has an average 6,8 non-radical value. According to Tetan et al. (2014), the higher the concentration is, the higher the inhibit zone.[8] This is suitable with Pelzcer and Chan's opinion, where the higher the concentration of antibacterial material, the stronger its antibacterial activity.

In this research, the rimpang bangle extract can hamper the growth of bacteria *Staphylococcus aureus,* or bacteriostatic. The concentration influences the diffusion of useful substances. The higher the concentration is, the faster the diffusion. The result also shows stronger inhibition of antibacterial and wider its forming obstacle diameter zone.[9]

Rimpang bangle extract can inhibit *Staphylococcus aureus* because it contains several materials, such as saponin, flavonoid, atsiri oil, alkaloid, tannin, and glycoside. These additive substances have different mechanisms in holding up *Staphylococcus aureus.*[10]

Flavonoids act as bacteriostatic. It has a denaturalizing protein of bacteria cell working mechanism, and it can destroy cytoplasm membrane. Flavonoids work as an inhibitor that hampers the bacteria's DNA replication and transcription. It can tangle with the protein of extracellular bacteria and can dissolve bacteria cell walls.[11] Flavonoid compounds can destroy cytoplasm membrane resulting in the leaking out of crucial metabolism and deactivating enzyme system of bacteria. It can make nucleotide and amino acid infiltrates, avoid some active materials in the cell, and kill the bacteria.[12] According to Yuslianti et al. (2021) honey flavonoid compound has antibacterial effect.[13]

Tannin has antibacterial activity. The estimated mechanism is the toxicity of tannin-kill bacteria membrane cells. It can induce the complex form of tannin to the metal ion and increase tannin toxicity. Tannin can frown cell walls or membranes; Thus, it disturbs cell permeability.

Therefore, the cell is unable to do to activity; then it eventually die.[14,15]

The triterpenoid mechanism as an antibacterial is to react with porin (transmembrane protein) in the membrane of bacteria wall cells, forming a strong polymer bond, and damaging porin. The steroid compound in triterpenoid shows antibacterial activity, antifungal, antitumor, and neurotoxic. Quinone compound provides a source of stable free radicals and forms an irreversible complex with protein nucleofilament acid. Thus, it results in the deactivation of protein. Quinone binds polypeptides and bacteria enzymes.[12]

The results show unclear results in which substance has a crucial role in inhibiting the growth of *Staphylococcus aureus*. These active compounds can work in different ways or together in doing the activity.

CONCLUSION

Rhizome bangle extract with 32% concentration can inhibit the growth of *Staphylococcus aureus* in comparison with concentrations of 8% and 16% in vitro.

CONFLICT OF INTEREST

As a result, we declare no conflict of interest in the scientific articles we write.

ACKNOWLEDGMENT

Our thanks go to the professionals in Faculty of Dentistry University of Mahasaraswati Denpasar, Bali, Indonesia assisted in the research and preparation of the paper.

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