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The Anti-Bacterial Potency of Tamarind (*Tamarindus Indica* L.) Seed Coat Extract Against the Growth of *Staphylococcus aureus* ATCC® 29213™ (In Vitro)

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ABSTRACT

The prevalence of oral bacterial infections, such periodontitis caused by *Staphylococcus aureus* is a global issue affecting several countries, including Indonesia. However, the use of Tamarind (*Tamarindus indica* L.), a multifunctional plant with antibacterial, antidiabetic, anticholesterol, antioxidant, and analgesic effects, has proven to be effective overcome this issue. The antimicrobial compounds of tamarind such as polyphenols, tannins, and anthocyanins, which damage cell walls, inactivate enzymes, and interfere with protein transport, involve bacteria lysis. Therefore, this study aims to determine the antibacterial effect of tamarind seed coat extract against *Staphylococcus aureus* ATCC® 29213™. The design of this study is experimental laboratory, with a post-test-only control group design, which was analyzed using ANOVA and Least Significant Difference (LSD) tests. The parameters measured included the zone of inhibition, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) at various extract concentrations of 15%, 10%, 5%, and 2.5%. The inhibition zone was determined using the blank disk method with a digital caliper, while the MIC and MBC were measured using the dilution method. Tamarind seed coat was extracted using a maceration method with 70% ethanol, divided into four group concentrations. The results showed average inhibition zones of 15 mm, 12.7 mm, 10.6 mm, and 0 mm at concentrations of 15%, 10%, 5%, and 2.5%, respectively. The MIC and MBC obtained through the dilution method in the tube were clearer at 5% and turbid at 15%. Furthermore, the value of both parameters at 5% and 10% were determined by subculturing the solution onto Mueller Hinton Agar (MHA) media. Based on the results, higher concentrations of the extract were more effective against *Staphylococcus aureus* ATCC® 29213™.

Key words: Tamarind seed coat, *Staphylococcus aureus* ATCC® 29213™, inhibition zones,

ABSTRAK

Peranan bakteri penyebab infeksi rongga mulut merupakan permasalahan global yang terjadi saat ini dan menjadi persoalan besar bagi masyarakat Indonesia, terutama periodontitis disebabkan bakteri *Staphylococcus aureus*. Asam jawa (*Tamarindus indica* L.) merupakan tumbuhan multifungsi yang banyak ditemukan di Indonesia sebagai antibakteri, antidiabetes, antikolesterol, antioksidan dan analgesik. Ekstrak kulit biji asam jawa memiliki senyawa sebagai antimikroba yaitu polifenol, tanin dan antosianin yang dapat merusak dinding sel, menginaktivasi enzim, mengganggu transpor protein menyebabkan bakteri akan *lysis*. Tujuan penelitian adalah untuk mengetahui zona hambat, nilai Kadar Hambat Minimum

(KHM) dan Kadar Bunuh Minimum (KBM) ekstrak kulit biji asam jawa pada konsentrasi 15%, 10%, 5% dan 2,5% terhadap *Staphylococcus aureus* ATCC® 29213™. Metode yang digunakan eksperimental laboratoris dengan rancangan penelitian *post test only control group design* dilakukan analisa uji ANOVA dan LSD. Penentuan zona hambat menggunakan metode difusi menggunakan cakram *(blank disk)* yang diukur menggunakan kaliper sorong digital, sedangkan KHM dan KBM dipakai metode dilusi. Kulit biji asam jawa diekstrak dengan metode maserasi menggunakan etanol 70%, dikelompokkan ke dalam 4 perlakuan dengan berbagai konsentrasi, dilakukan replikasi 4 kali. Hasil penelitian didapatkan zona hambat dari konsentrasi 15%, 10%, 5% dan 2,5% dengan rata-rata 15 mm, 12,7 mm, 10,6 mm dan 0 mm. Nilai KHM dan KBM pada metode dilusi pada tabung terlihat lebih jernih pada 5% dan pekat pada 15%, dilanjutkan dengan subkultur larutan ke media *Mueller Hinton Agar* (MHA) sehingga mendapatkan nilai KHM dan KBM yaitu pada konsentrasi 5% dan 10%. Kesimpulan bertambah tinggi konsentrasi membuktikan efektivitas ekstrak kulit biji asam jawa yang lebih baik terhadap bakteri *Staphylococcus aureus* ATCC® 29213™.

Kata Kunci: Kulit biji asam jawa, *Staphylococcus aureus* ATCC® 29213™, zona hambat, KHM dan KBM.

1. Introduction

The Bacteria's role in causing oral infection is a global problem that frequently occurs nowadays and has become a major issue in Indonesia, such as periodontitis. Colonization of microorganisms can damage the periodontal ligament and alveolar bone over time, as well as tooth loss if not treated properly. Microbe interactions cause damage to the host, so as to result variety of clinical symptoms and signs.[1],[2],[3]

The oral cavity is an essential component of human life. The oral moisture properties, nutrients and environment proper habitat for the growth of several microflora, such as *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus sp.*, and *Pseudomonas aeruginosa*. Normal bacterial growth in the mouth might become pathogenic as a result of various factors such as oral hygiene, temperature, pH, oxidation-reduction potential, nutrient availability, water, anatomical structure, salivary flow, and antimicrobial substances.[4],[5],[6]

Staphylococcus aureus is a normal flora in the oral cavity, whom can become a pathogen and cause infection because the changes of various factors. The bacteria anatomical is round shape, single, pairs, tetrads, or groups, and arranged like grapes shape on microscope. The microorganisms are facultative anaerobes, require organic nitrogen (e.g. amino acids) for growth. *Staphylococcus aureus* could be a serious problem because of its antibiotic resistance caused by improper drug use.[7],[8],[9]

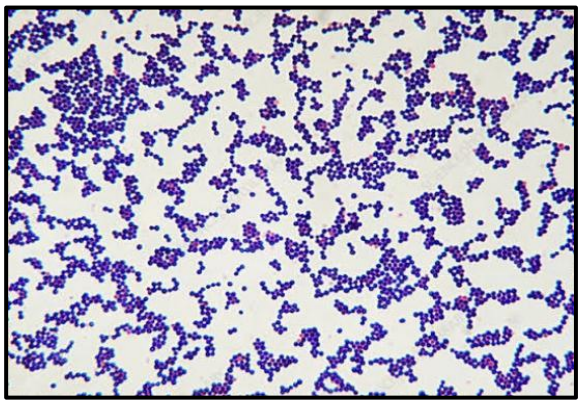

Fig 1. *Staphylococcus aureus* ATCC® 29213™

Staphylococcus aureus infection is characterized by tissue damage and purulent abscess. *Staphylococcus aureus* can cause boils, acne, impetigo, wound infections, as well as pneumonia, mastitis,

phlebitis, meningitis, urinary tract infections, osteomyelitis, and endocarditis. This bacterium become main role in nosocomial infections, food poisoning, and toxic shock syndrome. Gingivitis, abscess, angular cheilitis, parotitis, staphyloccal mucositis, denture stomatitis, staphyloccal osteomyelitis, chronic osteomyelitis, epulis, stomatitis, and dentoalveolar abscess can occur in the oral cavity caused the bacteria.[10],[11],[12],[13]

Tamarindus indica L., also known as tamarind, is a multifunctional plant native to Indonesia. This plant is frequently used in everyday life, such as a foods ingredients, traditional herb, and cosmetics materials. The significance of this research into flora as an alternative treatment that *Staphylococcus aureus* infection can be treated with antibiotics, but some people are allergic to drugs like amoxicillin, tetracycline, and others.[14],[15],[16]

Fig 2. Tamarind seed.

Previous research has shown that tamarind seed coat extract, at a concentration of 12.5%, can inhibit the growth of *Staphylococcus aureus*. Other studies have shown that tamarind seed coat extract can inhibit the growth of *Staphylococcus aureus*, with an average inhibition zone of 9 mm and a MIC value of 5.12%.[17],[18]

According to description above, the tamarind seed coat has antibacterial properties because it's compound such as tannins, polyphenols, and flavonoids; however, only been a few studies on the impact of the tamarind seed coat on the growth of *Staphylococcus aureus*. The author is interested in researching the inhibition zone, MIC and MBC of *Staphylococcus aureus* ATCC® 29213™ against tamarind seed coat extract because this bacterium is part of the normal flora in the oral cavity and can be pathogenic for a variety of infections. Tamarind seed coat extract is expected to be used clinically in dentistry as an alternative for oral cavity prevention and treatment.[19],[20]

2. Materials and Methods

Tamarind seed coat, Etanol 70%, *Mueller Hinton Agar, Nutrient Broth, Staphylococcus aureus* ATCC® 29213™, and DMSO.

2.1. Sample collection

The tamarind was collected by purposive sampling and obtained from along Tri Dharma street, 4th gate of Sumatera Utara University.

2.2. Laboratory equipment and personal protective equipment (PPE)

Digital weigh, paper disk, filter paper, cotton, plastic pot, percolator, erlenmayer, vial, vaccum rotavapor, vortex, incubator, micro pipette, ose, spiritus, petri dish, lab coat, mask and gloves.

2.3. Study type and design

The sort of exploration is in vitro trial research facility with posttest just benchmark group plan.

2.4. Study site and period

From September 2021 to March 2022, this study was carried out at the University of Sumatera Utara Hospital's Microbiology Laboratory.

2.5. Plant extract methods

Tamarind about 500 gr were washed with water and separate the flesh and seeds of the fruit. The seed of tamarind were dried in a drying cabinet for one week at temperature 50-60ºC, then mash and separate the seeds core and coat the tamarind seed coat then crushed into smooth pieces by blender (simplicia).

For the first six hours, 50 gr of simplicia was added to 1.5 liters of 70% ethanol and stirred. Refrigerate for 18 hours, occasionally stirring. The extract was then filtered and evaporated using a rotary vacuum Rotavapor at a temperature of 40ºC, and concentrated extract obtained, which was then placed in a vial.

2.6. Experiment group

This study used 6 treatment groups, each consisting of: Group I (15% tamarind seed coat extract), Group II (10% extract), Group III (5% extract), Group IV (2,5% extract), Group V (*Chlorhexidine* 2% as positive control), Group VI (DMSO as negative control).

2.7. Culturing, collection and preparation of the bacteria

A pure culture of *Staphylococcus aureus* ATCC® 29213™ from the University of Sumatera Utara Hospital's microbiology laboratory was used for the samples.

2.8. Inoculum procedure

An ose of *Staphylococcus aureus* ATCC® 29213™ was taken, then culture and suspend it in 0,9% NaCl 10 mL solution until the turbidity is the same as 0.5 McFarland standard. Put as much as 1 ose into the media and then streak / scrape tightly on the surface of the MHA on the petri dish.

2.9. Disk diffusion method

Antibacterial test using the diffusion method disc to determine the zone of inhibition (Kirby-Bauer). The principle of this method is to determine the agent's antimicrobial ability by measuring the diameter, which results in the antimicrobial strength inhibiting and even killing the test microbes. The formed inhibition zone is a colony-free area measured with a califer measure the vertical and horizontal diameters.[21]

Set sterile paper disc and dip them with tamarind seed coat extract with concentrations (15%, 10%, 5%, and 2,5%), while positive control with 2% chlorhexidine and negative with DMSO.

Place an ose by used sterilized cotton wood of *Staphylococcus aureus* ATCC® 29213™ on the petri disk and streak/scrape tightly on the surface of the MHA. Press the prepared paper disks gently onto the MHA medium in a petri dish with tweezers. Incubate for 24 hours at 37°C.

2.10. Dilution method

Label the test tubes and add 1 ml of NB media to each one to begin preparation. Drop 1 ml of various concentrations of tamarind seed coat extract (15%, 10%, 5%, and 2,5%) into each tube using a micropipette. in addition to 1 ml of each positive and negative control solution Using a cotton swab, insert one bacterial

streak in the inhibition zone of each concentration on a petri dish into each tube that has been vortexed to see the MBC. The tube's then incubated for 24 hours.

Each test solution was planted on MHA media and incubated for 24 hours to ensure the MIC and MBC values. The number of colonies was then determined using a colony counter. The lowest concentration in media that is not overgrown with bacteria is referred to as MBC.

2.11.Data analysis

Statistical Packaging for the Social Sciences (SPSS) was used to conduct the descriptive test, one-way ANOVA, and post hoc LSD analyses on the collected data.

3. Results

3.1. Diffusion-based testing of antibacterial potential

The *Staphylococcus aureus* ATCC® 29213TM was used to test the tamarind seed coat extract's antibacterial potential in a medium that had been inoculated with bacteria using the diffusion method. The inhibitory zone that formed around the discs is measured with a caliper with a precision of 0.02 and the average is obtained after 24 hours of incubation at 37°C.

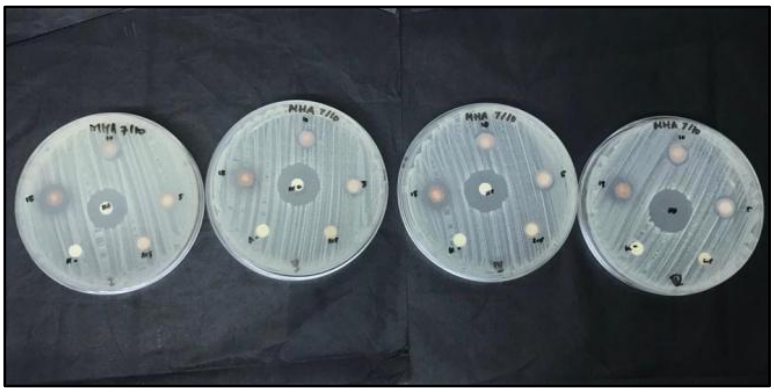

Fig 3. The formed inhibition zone of 2,5%, 5%, 10%, 15%, 2% chlorhexidine and DMSO.

Table 1. Average diameter of the *Staphylococcus aureus* ATCC® 29213TM inhibition zone created by tamarind seed coat extract at concentrations of 15%, 10%, 5%, 2,5%, *chlorhexidine*, 2%, and DMSO

Group	Inhibitory Diameter (mm) / Deuteronomy				
	I	II	III	IV	$\underline{x} \pm SD$
Extract 15%	16	14.5	15.25	14.25	15.00 ± 0.79
Extract 10%	13.5	12.25	13	12.25	12.69 ± 0.52
Extract 5%	10.25	10.25	10.75	11	10.56 ± 0.38
Extract 2,5%	0	0	0	0	0.00 ± 0.00
Control +	21.5	21.25	22	22.25	21.75 ± 0.46
Control -	0	0	0	0	0.00 ± 0.00

The results showed that the average diameter of the tamarind seed coat extract inhibition zone at 15% was 15.00 ± 0.79 mm, 10% was 12.69 ± 0.52 mm, 5% was 10.56 ± 0.38 mm, and was no inhibition at 2.5% concentration against *Staphylococcus aureus* ATCC® 29213™. The average inhibitory diameter in the 0.2% *chlorhexidine* as positive control group was 21.75 ± 0.46 mm. There was no inhibition zone in the DMSO as negative control group. According to the findings of this study, the concentration of 15% produced the best inhibition zone against *Staphylococcus aureus* ATCC® 29213™ by tamarind seed coat extract.

Using the statistical test Shapiro Wilk, all of the research data on the antibacterial potential of tamarind seed coat using the diffusion method were checked for normality. The normal distribution of the data was established by the results of the normality test. Therefore, the one-way ANOVA and post hoc LSD can be utilized in this study's data analysis.

The statistical test yielded a p value of 0.001 (p 0.05). This value demonstrates that the concentrations of tamarind seed coat extract—15 percent, 10 percent, 5 percent, 2,5 percent, chlorhexidine, and DMSO inhibit the growth of *Staphylococcus aureus* ATCC® 29213™ in significantly different ways. According to the statistical results, tamarind seed coat extract has the ability to inhibit the bacteria *Staphylococcus aureus* ATCC® 29213™. This means that the level of inhibition against *Staphylococcus aureus* ATCC® 29213™ increases with the concentration of tamarind seed coat extract.

The difference in the antibacterial potency of the tamarind seed coat extract at concentrations of 15%, 10%, 5%, 2,5%, chlorhexidine 0,2%, and DMSO in inhibiting the bacteria *Staphylococcus aureus* ATCC® 29213™ was determined using the one-way ANOVA test and post hoc LSD.

3.2. Dilution-based testing of antibacterial potential

The MIC value could not be determined because the tamarind seed coat extract produced a thick, dark color in this study, making it difficult to determine the level of clarity of the liquid media. The scientist then kept establishing on strong media (MHA) to have the option to see number of provinces that developed and determined from every grouping of the tried concentrate to get the worth of MBC. Planting on solid media revealed that the concentrations of 15% and 10% of the tamarind seed coat extract failed to promote the growth of the bacterium *Staphylococcus aureus* ATCC® 29213™.

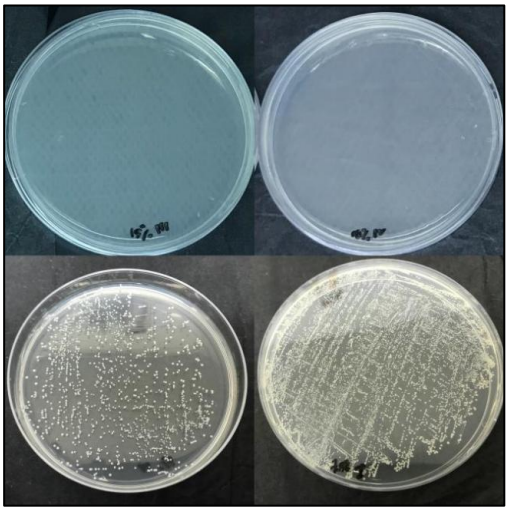

Fig 4. Counting colony of *Staphylococcus aureus* ATCC® 29213™ on plate disk.

Table 2. Average number of bacterial colonies *Staphylococcus aureus*

ATCC® 29213™ in each treatment group.

Group	The Number of Colonies (CFU/ml)				
	Repetition				
	I	II	III	IV	$\bar{x} \pm SD$
Extract 15%	0	0	0	0	0
Extract 10%	0	0	0	0	0.00 ± 0.00
Extract 5%	236	246	293	278	263.25 ± 26.73
Extract 2.5%	312	334	312	320	319.50 ± 10.38
Control +	0	0	0	0	0
Control -	365	387	369	366	371.75 ± 10.31

Table 2 shows that when tamarind seed coat extract concentrations of 2,5% were administered, the average number of bacterial colonies that grew was 319.50 10.38 CFU, and the concentration of 5% was 263.25 26.73 CFU, while no bacterial colonies grew at 15% and 10% concentration. This study found that at a concentration of 10%, tamarind seed coat extract had an MBC value against the bacteria *Staphylococcus aureus* ATCC® 29213™. Colonies grew in the negative control group (DMSO) with an average of 371.75 10.31 CFU, but there were no colonies in the positive control group (chlorhexidine).

Using the statistical test Shapiro Wilk, all of the research data on the dilution method's antibacterial potential of tamarind seed coat were examined for normality. The normal distribution of the data was established by the results of the normality test. Therefore, the one-way ANOVA and post hoc LSD can be utilized in this study's data analysis. This dilution method's statistical test, a one-way ANOVA, aims to determine the difference in the test material's antibacterial potential for killing the growth of the bacteria *Staphylococcus aureus* ATCC® 29213™.

The ANOVA and LSD tests showed significant results in each. This shows that tamarind has the ability to inhibit and eliminate *Staphylococcus aureus* ATCC® 29213™. Therefore, this research showed that tamarind extract is efficient in inhibit and eliminate the growth of *Staphylococcus aureus* ATCC® 29213™.

4. Discussion

Based on the results of the research conducted, the results obtained from the Minimum Inhibitory Concentration (MIC) with a concentration of 5% resulted in the growth of bacterial colonies with an average number of 237.25 CFU/mL, while the Minimum Bactericidal Concentration (MBC) was 10% with an average of 0 CFU/mL. The results of the diffusion method give the highest average diameter of the inhibition zone of 15 mm at a concentration of 15%, which is based on the criteria of the antibacterial power of Davis and Stout with the diameter of the inhibition zone being categorized as strong with a range of 10-20 mm. This proves that the extract of the tamarind seed coat has antibacterial activity due to the content of active compounds, namely tannins, polyphenols, and anthocyanins.[22]

The choice of the positive control in this study was Chlorhexidine 0.2% because it became the gold standard for preprocedural mouth rinse. Chlorhexidine gluconate 0.2% is the most common preparation in dentistry as an antiseptic.[23]

The antibacterial activity test using the dilution method followed by subculture on solid media was carried out because, in the preliminary test using the tube method, turbidity between concentrations could not be observed because all the colors of the tube were cloudy when compared to the control group. Turbidity is influenced by the color of the extract, which is concentrated and dark so that in direct visual observation, the level of turbidity of each concentration cannot be observed.

There are similarities in the effectiveness of the antibacterial power of tamarind seed coat extract in the results of the previous study. Wandee et al. (2022) prove that tamarind seed coat extract has antimicrobial activity to *Staphylococcus aureus* with MBC at 3,9% and MIC 0,09%. The difference in the values of MIC, MBC, and inhibition zone with previous researchers is due to factors, namely the type of solvent used and the place of plant physiological growth, that affect elemental content of plant compounds.[24],[25],[26],[27]

In this study, it was proved that tamarind seed coat extract has potential effectiveness against *Staphylococcus aureus* ATCC® 29213™ bacteria. This happened because the tamarind seed husk extract (*Tamarindus indica* L.) could inhibit growth at a concentration of 5% and kill at a concentration of 10% against *Staphylococcus aureus* ATCC® 29213™.

The choice of the best concentration is 10% compared to 15% for the MBC value based on the effect of higher toxicity at higher concentrations. The greater the concentration, the greater the toxic effects that may arise due to its use in the body. This is due to the pharmacodynamic mechanism because the reaction between the extracted phytochemical compounds with substances and nutrients from other foods consumed does not occur perfectly and can cause various problems. Chemical interactions between plant extracts and substances consumed from other foods have a risk if consumed beyond the body's tolerance limit. [27],[28],[29],[30]

5. Conclusion

The best inhibition zone was at a concentration of 15% with a width of 15.00±0.79 mm, and the MBC was 10% while the MIC was 5%. The concentration of 10% can break the colony of *Staphylococcous aureus.* The higher concentrations resulted in an increase in effectiveness of the tamarind (*Tamarindus indica* L.) seed coat extract.

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