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ANTIBACTERIAL TESTING OF MORINGA OLEIFERA LEAF EXTRACT AGAINST STREPTOCOCCUS MUTANS ATCC® 25175™

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Abstract

This study evaluates the antibacterial activity of Moringa oleifera leaf extract against Streptococcus mutans ATCC® 25175™. Leaf extracts from mountainous and coastal areas were tested using maceration with 96% ethanol, dilution for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), and disk diffusion for inhibition zones. Results showed that the mountainous extract was more effective, with a maximum inhibition zone of 18.38 mm and MBC of 100%, compared to the coastal extract with an inhibition zone of 12.94 mm and MBC of 75%. This study highlights the potential of Moringa oleifera leaf extract as a natural antibacterial agent for mouthwash development.

Keywords: *Moringa oleifera, Streptococcus mutans*

INTRODUCTION

Plants with medicinal potential offer numerous benefits to humans due to the presence of various active compounds, making them valuable for therapeutic purposes (Marhaeni, 2021). Utilizing plants as medicine is an alternative for disease prevention and treatment, as it is considered to have fewer side effects and can help reduce antibiotic resistance (Savitri et al., 2018). The rise in antibiotic resistance creates an opportunity to discover antibacterial compounds from medicinal plants (Munirah, 2020; Adiyasa, 2021).

One such plant is Moringa oleifera, known for its antibacterial or antimicrobial properties (Zubair, 2020). The active compounds responsible for these effects come from secondary metabolites, which are influenced by the plant's growing environment (Zaffer., 2020). Savitri's (2018) study demonstrated that the ethanol extract of Moringa leaves can inhibit the growth of Staphylococcus aureus, while Tarigan (2020) found it effective against Streptococcus mutans, a bacterium that causes dental infections. Similarly, Maghfiroh (2022) confirmed the inhibition of Streptococcus mutans by Moringa leaf extract through in vitro testing.

Streptococcus mutans is an anaerobic gram-positive bacterium that contributes to dental caries by producing acids that lead to tooth demineralization (Putra & Rahayu, 2017). The bacterium is prevalent in the oral cavity and is classified into serotypes, with serotype c being the most common at 70-80% (Matsumoto & Nakano, 2014; Annisa, 2015). Moringa leaf extract shows promising potential as an antibacterial agent, particularly against Streptococcus mutans. In this study, the Moringa leaves were sourced from the Seulawah Agam mountains in Aceh Besar to examine the active compounds that influence their antibacterial activity. Despite its potential,

Muhammad Ichsan[1], Cut Soraya[2], Zaki Mubarak[3], Syahrun Nur[4], M. Riza Pahlevi[5]*

Moringa leaf extract has not yet been utilized as an ingredient in mouthwash. Therefore, this research aims to develop a mouthwash containing ethanol extract of Moringa leaves, with antibacterial activity against Streptococcus mutans ATCC® 25175™ being tested in vitro.

LITERATURE REVIEW

Streptococcus mutans is a gram-positive facultative anaerobic bacterium in the S. mutans group, including S. sobrinus and others. Serotype c is most common in the oral cavity (70-80%), followed by serotype e (20%), while serotypes f and k are less than 5%. These bacteria cause dental caries (Matsumoto-Nakano, 2014). The oval shape and Gram stain results distinguish S. mutans. This bacterium is nonmotile and grows optimally at 18°-40°C (Hurst, 2019).

The structure of S. mutans includes a cell wall of peptidoglycan and antigens such as proteins, polysaccharides, and lipoteichoic acid. Its virulence includes the ability to produce organic acids, survive in low pH, and form glucan from sucrose for colonization of dental biofilms (Lemos et al., 2019). S. mutans produces glucosyltransferase (GtF) enzymes for attachment and biofilm formation, including GtF B, GtF C, and GtF D that form glucan from sucrose, increasing adhesion and proportion of dental plaque (Gao, 2021).

Biofilm formation starts from interactions between planktonic bacteria and oral surfaces in response to environmental signals (Bjarnsholt et al., 2018). S. mutans metabolizes carbohydrates to form biofilms on teeth, allowing tolerance to environmental fluctuations such as nutrient availability and pH changes (Bedoya-Correa, 2019). Quorum sensing is used to regulate physiological processes based on cell density, through two-component signal transduction systems (TCSTS) (Junges et al., 2019). Dental plaque formation as a biofilm is influenced by adhesion, nutrient flow, and coaggregation, which affect biofilm growth, gene expression, and virulence. The interaction between bacterial adhesins and receptors on the teeth initiates biofilm formation (Bedoya-Correa, 2019).

S. mutans adheres to teeth via sucrose-independent adhesion to salivary components for initial attachment, and sucrose-dependent adhesion for colonization. Sucrose consumption affects bacterial colonization, and transmission from the mother is the main source of colonization in infants. Glucan synthesis by S. mutans enhances adhesion and triggers dental caries (Bedoya-Correa, Rodríguez, and Parada-Sanchez, 2019). S. mutans produces acid rapidly, which is associated with dental caries. Acidity alters biofilm ecology, increasing S. mutans and other acidogenic bacteria that are acid-tolerant, affecting biofilm virulence in causing dental caries (Li et al., 2020).

Moringa (Moringa oleifera) leaves are known for their benefits in food, medicine, and industry. Originating from the Himalayas, these leaves have antioxidant properties that protect cells from free radical damage and prevent oxidative damage from a high-fat diet (El-Shehawi et al., 2021). Moringa seed extract shows anti-inflammatory and anti-microbial activity against bacteria and fungi (Fouad, 2019; Bancessi et al., 2020). Research shows that moringa leaf methanol extract is effective as an antibacterial agent against several pathogenic bacteria (Abadallah and Ali, 2019).

METHOD

Research Location and Time

This study was conducted in March 2022 at the Faculty of Veterinary Medicine laboratory, Universitas Syiah Kuala.

Tools and Materials

Tools used include an analytical balance (Radwag/AS 220), rotary evaporator (Hidolph Laborota 4003 Control), microscope (Olympus), incubator (Isuzu), Whatman No. 41 filter paper, glassware (Pyrex), petri dishes, and other laboratory instruments. Materials include Streptococcus

Muhammad Ichsan[1], Cut Soraya[2], Zaki Mubarak[3], Syahrun Nur[4], M. Riza Pahlevi[5]*

mutans ATCC 25175 bacteria, 96% ethanol, ethyl acetate, concentrated ammonia, methanol, and n-hexane.

Sample Collection

Moringa leaves were collected from Seulawah Agam Mountain, Aceh Besar, totaling ±5 kg.

Procedures

a) Moringa Leaf Preparation and Extraction
The leaves were cleaned, air-dried for seven days, and ground into powder. Then, 100g of the powder was macerated in 1000 mL of 96% ethanol for three days, stirred daily, and filtered. The extract was concentrated using a rotary evaporator for antibacterial testing against Streptococcus mutans.

b) Antibacterial Activity Test
A gel formulation containing Moringa extract was prepared by combining a gelling agent, preservatives, and penetration enhancers, then adding the extract.

c) Streptococcus mutans Growth Inhibition Test
Streptococcus mutans ATCC 25175 was cultured and standardized to McFarland 0.5 (1.5x10^8 CFU). Moringa extract was added to a 96-well plate with bacterial solution, incubated for 24, 48, and 72 hours, and bacterial growth was measured using a spectrophotometer at 620 nm.

RESULTS AND DISCUSSION

Antibacterial testing was performed using two common methods: the dilution method to observe the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), and the disk diffusion method to observe inhibition zones. The antibacterial activity of the ethanol extract of Moringa oleifera leaves from coastal and mountainous regions against Streptococcus mutans was tested using the Kirby Bauer disk diffusion method with Streptococcus mutans ATCC 25175 and a McFarland standard of 0.5. The results are shown in sub chapter 3.1.

Sub Chapter 3.1. Inhibition Zone Test Results

The ethanol extract from coastal regions, at concentrations of 6.25%, 12.5%, and 25%, exhibited moderate activity with inhibition zones between 5–10 mm, while concentrations of 50% and 75% showed strong activity with zones of 10–20 mm. The ethanol extract from the mountainous region at 6.25% concentration showed moderate activity, while at 12.5% and above, the results indicated strong activity. Overall, the ethanol extract from the mountainous region was more effective against Streptococcus mutans than the coastal region extract.

The antibacterial inhibition of the Moringa oleifera leaf extract was further evaluated by determining MIC and MBC against Streptococcus mutans. The results are presented in sub chapter 3.2.

Sub Chapter 3.2.

MIC and MBC Results

- Mountainous: MIC = 75%, MBC = 100%
- Coastal: MIC = 50%, MBC = 75%

Sub Chapter 3.3 demonstrates that all concentrations of Moringa oleifera ethanol gel extract (3.125%, 6.25%, 12.5%, 25%) reduced Streptococcus mutans growth. Incubation times of 24 and 48 hours showed the most significant reductions, with OD <0.1 (<300 CFU/mL). At 72 hours, only the 6.25% and 3.125% concentrations had better effects compared to others. The positive control (CHX) remained stable at both 24 and 48 hours.

Muhammad Ichsan[1], Cut Soraya[2], Zaki Mubarak[3], Syahrun Nur[4], M. Riza Pahlevi[5]*

Sub Chapter 3.3. Distribution and Frequency of Streptococcus mutans Growth Post- Gel Application

The Kruskal-Wallis analysis showed significant differences in Streptococcus mutans growth influenced by the Moringa oleifera ethanol gel ($p < 0.05$, $r = 0.653$), depending on incubation time. However, the concentration at each time point did not show significant differences. All concentrations exhibited bacteriostatic effects for 24, 48, and 72 hours, indicating that the extract could degrade Streptococcus mutans cell function during growth adaptation. This antibacterial ability is linked to several compounds in Moringa oleifera leaves, such as quinic acid, which plays a role in cell toxicity (Savitri et al., 2018; Soraya, 2022).

Recent studies by Gani (2022) also found that the antibacterial activity of Moringa oleifera extract against Streptococcus mutans increased with incubation time, particularly up to 24 hours. Additionally, incubation time can influence the extract's effect on the virulence of Streptococcus mutans (Sivakami et al., 2021). Several studies suggest that Moringa oleifera extract significantly inhibits the production of extracellular polysaccharides and reduces the expression of virulence-related genes in Streptococcus mutans after 24 and 48 hours of incubation (Buyela, 2017; Sivakami et al., 2021).

CLOSING

Conclusion

In this study, the results of the three inhibition zone tests against Streptococcus mutans showed that the Moringa leaf extract from the mountainous region was more effective than the extract from the coastal area. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results from the tables above also indicated that the Moringa leaf extract from the mountains was superior to the coastal extract.

The antibacterial testing of Moringa leaf extract with various concentrations (3.125%, 6.25%, 12.5%, 25%) was able to reduce the growth of Streptococcus mutans. All concentrations tested in this study exhibited excellent bacteriostatic effects over 24, 48, and 72 hours. This phenomenon suggests that the Moringa leaf extract can degrade the function of Streptococcus mutans cells during the adaptation phase, as supported by previous research mentioned in the discussion above.

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