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Research Report

Antibacterial efficacy of *Mangifera indica L.* leaves extract against *Streptococcus mutants*

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ABSTRACT

Background: Various preventive actions are being developed to prevent dental caries. On the other hand, some of these methods can only be applied by the dentist. Therefore, it is necessary to find an alternative caries prevention method. **Purpose:** This study aimed to determine the antibacterial activity of mango (Mangifera indica L.) leaves extract in inhibiting the growth of *Streptococcus mutans*. **Methods:** This research is experimental laboratory research and used a random sampling technique. Mango (*M. indica L.)* leaves extract was diluted using the double dilution method, which were 50%, 25%, 12.5%, 6.25%, 3.12%, 1.6%, 0.8%, 0.4%, 0.2%, 0.15%, 0.1%, 0.05%, and 0.025%. The Minimum Bactericidal Concentration (MBC) of mango (*M. indica L.)* leaves extract against *S. mutans* was determined using the agar plate thinning method. **Results:** The results showed no bacterial growth in the media with 100%, 50%, and 25% mango leaves extract. In addition, it was found that in media with 12.5% mango leaves extract still showed colony growth. **Conclusion:** Mango (*M. indica L.)* leaves extract in ethanol solvent has an antibacterial effect against *S. mutans* with the MBC value of 25%.

Keywords: dental caries; mango leaves extract; MBC; *Streptococcus mutans;* medicine

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INTRODUCTION

Dental caries is a common dental health problem in Indonesia. Based on the results of National Basic Health Research (RISKESDAS) in 2018, the prevalence of caries reached 88.8% in Indonesia. Dental caries is chronic and takes a long time to develop, so most sufferers have the potential to experience lifetime disorders. However, this disease often gets less attention from the community and health program planners.[1–3] Dental caries is caused by accumulated plaque that is not cleaned immediately. The dominant bacteria in dental plaques is the type of cocci, namely *Streptococcus mutans,* which can synthesize carbohydrates into extracellular polysaccharides and acids.[4]

Various preventive actions are being developed to control dental caries prevention in the community, including using antibacterial agents, pit and fissure sealants, vitamin D, fluoride applications, and diet control. However, some of these methods can only be applied by the dentist.[5] Meanwhile, Indonesian people have a kind of stigma that is "afraid of going to the dentist" and also needs high costs, making this prevention method less effective in Indonesia.[6] Therefore, it is necessary to find an alternative caries prevention method that can be easily applicable to larger communities, especially for Indonesians That alternative method is through the utilization of natural resources.

Mango (*Mangifera indica L.)* is a seasonal fruit that has become the agricultural sector's mainstay and is consumed locally in Indonesia. Indonesia is one of the largest mango producer countries, with production of 2.808.936 tons in 2019. Hannan et al.[7] stated that mango leaves extract has strong inhibitory power against pathogenic bacteria. In addition, the high concentration of mangiferin in mango leaves extract has various pharmacological activities, such as antioxidant, analgesic, antidiabetic, anti-inflammatory, antitumor, anti-HIV, and antibacterial. Meanwhile, according to Kumar et al.[8], mango leaves contain tannins in high concentrations which have potential as antioxidants. Furthermore, the mangiferin content in young leaves is higher than in old leaves. So in this study, young Gadung mango leaves were used as an extract.

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This research was conducted with the expectation that the results of this research could be helpful for the development of dentistry, so that mango leaves extract could be implemented in the preventive effort of dental caries. This study aimed to determine the antibacterial activity of mango leaves extract in inhibiting the growth of *S. mutans* and to determine the effective concentration of mango leaves extract in inhibiting the growth of *S. mutans*. Therefore, using mango (*M. indica L.)* leaves extract could be implemented as an alternative approach to maintain oral hygiene. Furthermore, This study aimed to determine the antibacterial activity of mango (*M. indica L.)* leaves extract in inhibiting the growth of *S. mutans*.

MATERIALS AND METHODS

This research is experimental laboratory research, which was conducted at the Laboratory of the Department of Pharmacognosy and Phytochemistry for the extraction of mango (*M. indica L.)* leaves, the Microbiology Laboratory of the Faculty of Dental Medicine, Universitas Airlangga to determine the antibacterial effect of mango leaves extract against *S. mutans*.

Mango (*M. indica L.)* leaves used in this study are light green leaves, ± 12 cm long, ± 4 cm wide, collected in Pandaan, Pasuruan, East Java, Indonesia. This study used a random sampling technique. The sample size for determining Minimum Bactericidal Concentration (MBC) values starts from tube 1 with 50% mango leaves extract to tube 12, which contains 0.05% mango leaves extract, followed by tubes 14 and 15, which contain positive controls and negative controls. The total number of samples was 45 samples with three replications each.

Mango (*M. indica L.)* leaves extraction procedure was carried out by mixing mango leaf powder with 700 ml of 70% alcohol until all fragments were submerged and then filtered with a Buchner funnel to obtain the extract in liquid form. The liquid extract was then evaporated until it was free from ethanol solvent using a rotary evaporator at 40ºC for 3 hours to become a 14 g thick extract. The extract was diluted with sterile distilled water and 2% Dimethyl Sulfoxide (DMSO).

The extract was then diluted using the dilution method. 100% Mango leaves extract solution, mixed with Brain Heart Infusion Broth (BHIB) media until the concentration becomes 50% (tube 1), 25% (tube 2), 12.5% (tube 3), 6.25% (tube 4), 3.12% (tube 5), 1.6% (tube 6), 0.8% (tube 7), 0.4% (tube 8), 0.2% (tube 9), 0.15% (tube 10), 0.1% (tube 11), 0.05% (tube 12), and 0.025% (tube 13).

The sensitivity test of *S. mutans* against mango (*M. indica L.)* leaves extract was carried out by taking 1 ml of Gadung mango leaves extract at a concentration of 100% and mixing it with 1 ml of BHIB and 0.1 ml of bacterial inoculum (1.5 x 10^8 CFU/ml). They were then incubated for 24 hours in 37ºC. The dilution results of mango leaves extract containing *S. mutans* and BHIB media (tubes 1-13), positive and negative control tubes were then incubated at 37ºC for 24 hours.

The MBC of mango leaves extract against *S. mutans* was determined using the agar plate thinning method. Bacterial culture in BHIB aqueous media was taken at 0.1 ml and cultured in Tryptone Yeast Cystine (TYC) media at 37ºC for 24 hours. After 24 hours of incubation, bacterial growth was observed and compared to the colonies' growth in the positive control. From the observations, it is possible to determine the MBC of mango (*M. indica L.)* leaves extract against *S. mutans*.

RESULTS

Mango (*M. indica L.)* leaves were washed thoroughly and then drained to remove the remaining washing water. Two hundred forty-eight grams of Mango leaves that had been dried and crushed were extracted, and a thick green-black extract was obtained (Figure 1), stored in a closed glass container, and placed in a cold place.

The results showed no bacterial growth (0 CFU/ml) in the media with 100%, 50%, and 25% mango leaves extract visible from the clear zone on the Petri dish (Table 1, Figure 2). It is indicated that all *S. mutans* died. So, the MBC of mango (*M. indica L.)* leaves extract against *S.mutans* is 25%. In addition, it was found that in media with 12.5% mango leaves extract still showed colony

Figure 1. Mango (*M. indica L.)* leaves extract.

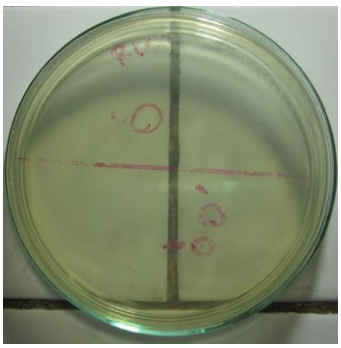

Figure 2. There was no bacterial growth in mango (*M. indica L.)* leaves extract media. The clear zone showed no growth of *S. mutans*.

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Table 1. Antibacterial activity of mango (*M. indica L.)* leaves extract against *S. mutans*

Groups	Replication 1	Replication 2	Replication 3
100% mango leaves extract	0 CFU/mL	0 CFU/mL	0 CFU/mL
50% mango leaves extract	0 CFU/mL	0 CFU/mL	0 CFU/mL
25% mango leaves extract	0 CFU/mL	0 CFU/mL	0 CFU/mL
12.5 % mango leaves extract		uncountable	
Positive control		uncountable	
Negative control	0 CFU/mL	0 CFU/mL	0 CFU/mL

Notes: 0 CFU/ml= Sterile, no bacterial growth; CFU/ml= Colony Forming Unit/ml

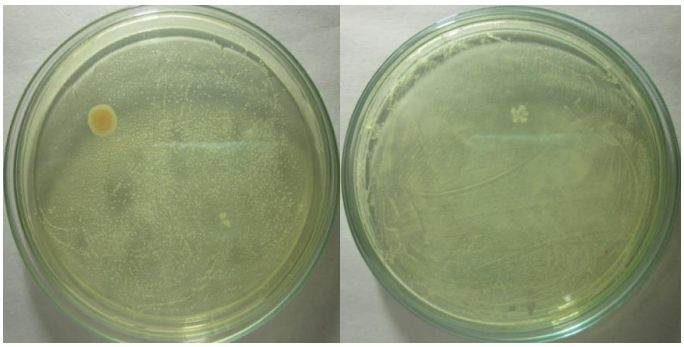

Figure 3. The number of *S. mutans* colonies in medium with mango (*M. indica L.)* leaves extract (left) and positive control (right) were uncountable.

growth (Table 1). However, the number of *S. mutans* colonies cannot be counted as well as the positive control (Figure 3).

DISCUSSION

In this study, the antibacterial effect of mango leaves extract was tested against *S. mutans*. Many kinds of laboratory methods can be used to determine an extract's in vitro antimicrobial activity. The most well-known, simple, and low-cost methods are the disk-diffusion and agar dilution methods. With this method, the test subject can make direct contact with microorganisms, so that the results obtained are more accurate, also the MBC values of the test subject can be investigated as recommended by National Committee for Clinical Laboratory Standards (NCCLS, USA).[9–11] The research was carried out using the double dilution so that the concentration of test subject was obtained which was half of the initial concentration, namely 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.6%, 0.8%, 0.4%, 0.2%, 0.15%, 0.1%, and 0.05%. At each concentration, replicated 3 times for more accurate results and to find out the average number of bacteria growths in mango (*M. indica L.)* leaves extract at various concentrations.

MBC is defined as the lowest concentration of the compound/extract without visible bacterial growth (apparent to the eye), resulting in no viable cells or 0 CFU/ml.[12] In this study, MBC was determined after being subcultured in TYC and incubated for 24 hours, no visible bacterial growth (sterile) was found at 100% concentration, as well as at 50% and 25% concentrations. However, at 12.5% concentration, bacterial growth was visible in the media. Therefore, the MBC value was determined as 25%.

The antibacterial properties in mango (*M. indica L.)* leaves extract is suspected because mango leaves extract has many active compounds. Mango (*M. indica L.)* leaves extract contains high concentrations of tannins and saponins, including numerous phenolic compounds such as gallic acid. Flavonoids quercetin and kaempferol, which have antibiotic effects, have also been found in mango leaves. Flavonoids can act directly as antibiotics by interfering with the function of microorganisms such as bacteria or viruses.[8,13–15] In addition to the several antibacterial compounds previously mentioned, mango leaves also contain mangiferin, which has the potential as an antibacterial agent.[16] Mangiferin is polyphenol substance which consists of 2 different aromatic chemicals, namely shikimate and ketate. This substance has been tested to have antibacterial activity, namely: *Escherichia coli, Klebsiella, Staphylococcus, and Pseudomonas*.[17]

Cell death in *S. mutans* may be induced by saponins that act as surfactants/soaps or detergents, which impair the bacterial cell boundary layer through the bonding of polar saponin groups with polysaccharides and peptidoglycan as well as non-polar saponin groups with lipoteichoic acid (LTA), causing disturbances in cell permeability, cell function, cell lysis and resulting in cell death.[18,19]

The action mechanism of flavonoids is suspected to damage cell membranes because of their lipophilic nature and ability to form complexes with extracellular proteins. Phenol compounds are toxic to microorganisms because they can inhibit important enzymes of microorganisms, thereby disrupting cell function and destroying protein compounds that can disrupt the semi-permeability of cell membranes.[20]

S. mutans consist of a cell wall and protoplasmic membrane, as in other gram-positive cocci. The cell wall

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matrix consists of cross-chain peptidoglycan and amino sugar N-acetyl, N-acetyl muramic acid, and several peptides. In addition, the antigenic structure of the cell wall of *S. mutans* consists of protein antigens, specific polysaccharides, and lipotechoic acid.[21] Therefore, inhibiting the growth of *S. mutans* requires a high concentration of mango (*M. indica L.)* leaves extract.

CONCLUSION

From the experimental research that has been done, it can be concluded that mango (*M. indica L.)* leaves extract in ethanol solvent has an antibacterial effect against *S. mutans* with the MBC value of 25%. However, further research is needed to determine which active ingredient has the most significant antibacterial effect on mango leaves; the MIC of mango (*M. indica L.)* leaves extract against *S.mutans*; and use mango (*M. indica L.)* leaves extract as an alternative drug to prevent dental caries in vivo so that this material can be used clinically.

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