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THE INHIBITORY EFFECT OF BAMBOO CHARCOAL EXTRACT ON THE GROWTH OF *STREPTOCOCCUS MUTANS*

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ABSTRACT

Background: *Streptococcus mutans* are the primary cause of dental caries due to their acidogenic and aciduric properties. Natural antibacterial agents, such as bamboo charcoal extract, have been explored as potential alternatives to synthetic antimicrobial agents. However, the effectiveness of bamboo charcoal extract in inhibiting *Streptococcus mutans* remains uncertain. This study aimed to evaluate the antibacterial potential of bamboo charcoal extract at various concentrations.

Method: This research was conducted using a laboratory experimental method. The number of samples per group was determined using the Federer formula $(t-1)(n-1) \geq 15$, where t is the number of treatment groups and n the number of replications. With seven groups (six concentrations of bamboo charcoal extract and one positive control), a minimum of three replications per group was required, resulting in 21 samples in total. Bamboo charcoal extract was prepared through maceration using 96% ethanol and tested at concentrations of 0%, 12.5%, 25%, 37.5%, 50%, and 62.5% against *Streptococcus mutans* using the disk diffusion method. A 0.2% chlorhexidine solution served as the positive control. Inhibition zones were measured after a 24-hour incubation at 37°C.

Result: Statistical analysis using descriptive analysis showed no significant difference in inhibition zones. None of the bamboo charcoal extract concentrations demonstrated antibacterial activity against *Streptococcus mutans*. In contrast, positive control (0.2% chlorhexidine) produced a significant inhibition zone of 12.56 mm.

Conclusion: Bamboo charcoal extract at concentrations of 12.5%, 25%, 37.5%, 50%, and 62.5% did not show any inhibitory effect on the growth of *Streptococcus mutans*.

INTRODUCTION

Bamboo charcoal has increasingly attracted attention in dental health research due to its antibacterial properties, whitening effect, and ability to reduce plaque accumulation. Lestari, Syamsurizal, and Trisna reported that activated charcoal toothpaste was effective in reducing plaque among smokers, suggesting its potential for oral hygiene.[1] The porous structure of bamboo charcoal

and its bioactive components, such as lignin and phenolics, may contribute to antibacterial activity by adsorbing bacterial metabolites and interfering with microbial adhesion. These advantages highlight bamboo charcoal as a promising natural alternative to synthetic antimicrobial agents for maintaining oral health and preventing dental caries.

Despite these promising characteristics, dental caries remains one of the most prevalent oral health problems worldwide, affecting individuals across all age groups. In Indonesia, the 2018 Basic Health Research (*Riskesdas*) reported that the prevalence of dental problems reached 57.6%, reflecting a significant public health burden.[2] If untreated, caries can lead to pain, infection, and broader systemic health issues, underscoring the need for effective preventive strategies.

The development of dental caries is closely associated with the activity of cariogenic bacteria, particularly *Streptococcus mutans*. This bacterium metabolizes dietary carbohydrates into acids, lowering the oral pH and promoting demineralization of the tooth enamel.[3,4] Given its central role in caries formation, inhibition of *Streptococcus mutans* growth has become a primary focus in caries prevention research.

Previous studies have indicated that natural extracts may exert antibacterial effects against oral pathogens. For instance, Minarni and Rosmalia demonstrated that pineapple core extract inhibited the growth of *Streptococcus mutans*,[5] while Maahirah and Saputri showed that bacterial biofilm formation could be affected by natural compounds.[6] However, evidence on the antibacterial effect of bamboo charcoal extract against *Streptococcus mutans* remains inconsistent, and the effective concentration required for inhibition has not been clearly established.

Based on this gap, the present study aimed to evaluate the inhibitory effect of bamboo charcoal extract at various concentrations (12.5%, 25%, 37.5%, 50%, and 62.5%) against *Streptococcus mutans*. The outcomes are expected to clarify whether bamboo charcoal extract has potential as a natural antibacterial agent for caries prevention.

Dental caries is caused by a complex interaction between cariogenic oral bacteria—primarily *Streptococcus mutans* and carbohydrates that adhere to the surface of the teeth. This process begins when the bacteria metabolize carbohydrates into acids, which then erode the tooth enamel. Acid production leads to demineralization, or the loss of essential minerals such as calcium and phosphate from the tooth structure. If the rate of demineralization exceeds that of remineralization (the natural repair process), the damage progresses into more severe caries.

Streptococcus mutans are the primary bacterium responsible for dental caries. Discovered in 1924 by J. Kilian Clarke, this bacterium belongs to the phylum Firmicutes. *Streptococcus mutans* can metabolize sucrose present in the oral cavity into glucose and fructose. Through glycolysis, glucose is further converted into pyruvate, producing energy in the form of ATP. Under anaerobic conditions,

pyruvate is transformed into lactic acid, which lowers the pH around the teeth, accelerates demineralization, and damages the tooth structure.

Preventing dental caries involves inhibiting the growth of *Streptococcus mutans*. Bamboo charcoal has demonstrated antibacterial properties that are effective against this bacterium. Research by Choi and Ahn showed that bamboo charcoal can eliminate *Streptococcus mutans*, suggesting its potential as an alternative for preventing or treating dental caries.[7] Bamboo charcoal is also known for its teeth-whitening effects and its ability to reduce plaque, both of which are important factors in the development of caries.

Although bamboo charcoal offers numerous benefits for oral health, research on the most effective concentration of bamboo charcoal extract to inhibit the growth and activity of *Streptococcus mutans* remains limited. Inhibition in this context refers not only to suppressing bacterial proliferation but also to reducing acid production and interfering with biofilm formation, which are critical factors in the cariogenic potential of *Streptococcus mutans*.[4,7] Previous studies have shown that bamboo charcoal possesses antibacterial effects through its porous structure, which can adsorb bacterial metabolites and disrupt bacterial adhesion to tooth surfaces[1] However, further studies are needed to determine the optimal concentration that consistently produces such effects, in order to ensure its effective application in dental care products. With its natural antibacterial properties and proven safety for oral use, bamboo charcoal shows promise as an alternative ingredient in dental treatments, though in-depth research is required to establish its most effective formulation.

RESEARCH METHODS

Tools and Materials

The equipment used in this study included: face masks, gloves, autoclaves, vortex, mixer, incubator, sliding caliper, pipette, pen, micropipette, sterile inoculating loop, petri dishes, a maceration setup (jars), filter paper, evaporating dish, cabinet dryer, beaker, microscope, tweezers, blender, and rotary evaporator. The materials used consisted of 96% ethanol, *Streptococcus mutans* strain ATCC 25175 (obtained from AGAVI Laboratory, Bandung), nutrient broth, Mueller Hinton Agar, bamboo charcoal (purchased via e-commerce), chlorhexidine gluconate 2%, and DMSO.

Preparation of Bamboo Charcoal Extract

All equipment was sterilized using an autoclave at 121°C for 15 minutes. A total of 1000 grams of bamboo charcoal was ground into a simplicial powder using a blender and sieved to obtain a particle size of 40 mesh. For the maceration process, 600 grams of the powder was divided into three batches, each weighing 200 grams. Each batch was soaked in 96% ethanol at a solvent-to-material ratio of 3:1

(v/w) in a beaker. The maceration process was carried out for 72 hours at room temperature in a dark environment to minimize degradation of active compounds. During this period, the mixture was stirred three times daily (every 8 hours) to ensure homogeneous extraction.

After 72 hours, the mixture from each batch was filtered using flannel cloth to separate the liquid extract (filtrate) from the solid residue. The collected filtrates were then combined and concentrated using a rotary evaporator at 60°C to remove ethanol. The semi-solid concentrate was further dried in a water bath to obtain a powdered bamboo charcoal extract, which was stored in airtight containers for subsequent antibacterial testing.

Media Preparation

A total of 38 grams of Mueller Hinton Agar powder was dissolved in 1 liter of distilled water and heated until boiling while stirring occasionally until a homogeneous solution was achieved. Then, 25 ml of the medium was poured into each petri dish.

Incubation Preparation

A bacterial suspension was prepared by adding nutrient broth to an inoculation tube, followed by the introduction of *Streptococcus mutans* colonies. The mixture was homogenized using a vortex mixer and adjusted to match the McFarland turbidity standard.

Antibacterial Activity Test

The bamboo charcoal extract was dissolved in dimethyl sulfoxide (DMSO) to obtain concentrations of 0%, 12.5%, 25%, 37.5%, 50%, and 62.5%. Petri dishes containing Mueller Hinton agar were prepared as the culture medium. *Streptococcus mutans* were inoculated by mixing one loopful of the bacterial suspension into 20 ml of medium, followed by even spreading using a figure-eight motion. Sterile paper discs were then impregnated with three drops of each extract concentration, as well as with the positive control (0.2% chlorhexidine solution), using a micropipette and placed on the surface of the inoculated agar. All plates were incubated at 37°C for 24 hours, after which the presence of clear zones (inhibition zones) surrounding the discs was observed and measured.

RESEARCH RESULT

Table 1. The results of measuring the inhibition zone for each treatment

NO	REPETITION	Positive Control	Negative Control	12,5%	25%	37,5%	50%	62,5%
1	1a	1.418	-	-	-	-	-	-
2	1b	1.555	-	-	-	-	-	-
3	2a	1.593	-	-	-	-	-	-
4	2b	1.538	-	-	-	-	-	-
5	3	1.528	-	-	-	-	-	-

This study showed that none of the treatments exhibited any inhibitory effect against *Streptococcus mutans*. The results indicated that only the positive control, 0.2% chlorhexidine, produced an inhibition zone measuring 1.256 cm², while no inhibition zones were observed in the treatment groups.

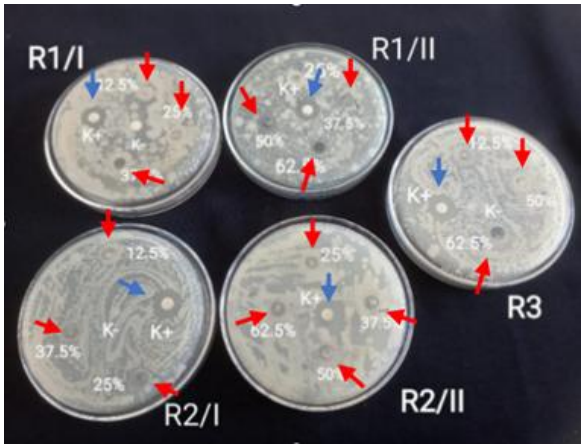

Figure 1. Results of inhibition test
Note: red (treatment), blue (positive control).

Red markers indicate treatment groups (bamboo charcoal extract at concentrations of 12.5%, 25%, 37.5%, 50%, and 62.5%), while the blue marker represents the positive control (0.2% chlorhexidine). The figure shows that no inhibition zones were formed around the treatment discs, whereas a clear inhibition zone was observed around the positive control disc, confirming the absence of antibacterial activity of bamboo charcoal extract against *Streptococcus mutans*.

DISCUSSION

The results of this study indicate that bamboo charcoal extract at all tested concentrations (12.5%, 25%, 37.5%, 50%, and 62.5%) did not produce inhibition zones against *Streptococcus mutans*. In contrast, the positive control (0.2% chlorhexidine) demonstrated a clear inhibition zone, confirming its effectiveness as a standard antimicrobial agent for oral pathogens. Statistical analysis further confirmed that there was no significant difference among the treatment groups ($p > 0.05$), suggesting that bamboo charcoal extract did not exhibit measurable antibacterial activity under the conditions tested.

These findings differ from several previous studies that reported antibacterial activity of bamboo-derived materials. Choi and Ahn demonstrated that bamboo charcoal had inhibitory effects against *Streptococcus mutans* in vitro,[7] while Lestari, Syamsurizal, and Trisna reported that activated charcoal toothpaste reduced plaque accumulation in smokers, indirectly indicating antibacterial potential.[1] Similarly, Fahira et al. found that ethanol extract of matoa leaves significantly inhibited *Streptococcus*

mutans growth, suggesting that natural plant-derived compounds generally have promising antimicrobial activity.[8]

The absence of antibacterial activity in the present study may be attributed to the carbonization process of bamboo charcoal. Bamboo contains phenolic compounds and lignin that exhibit antibacterial properties.[9] However, these compounds are heat-sensitive and degrade when bamboo is subjected to carbonization temperatures above 300°C for several hours.[10] As a result, the phenolic content of bamboo charcoal is greatly reduced compared to raw bamboo or its non-carbonized extracts, which explains the lack of inhibition zones observed in this study.

In addition to the loss of bioactive compounds, the solubility of bamboo charcoal extract in ethanol and DMSO may also influence its antibacterial performance. Activated carbon tends to act more as an adsorbent rather than as a direct antibacterial agent.[1] Its primary function is to bind toxins and metabolites, but without sufficient concentration of intact phenolic or other antimicrobial compounds, the extract may not exert significant inhibitory activity on bacterial growth.

Overall, the present study suggests that the antibacterial potential of bamboo charcoal is limited when prepared through conventional carbonization and ethanol maceration. Alternative extraction techniques, such as lower-temperature pyrolysis, nanoparticle formulations, or combining bamboo charcoal with other plant extracts rich in bioactive compounds, may be necessary to enhance its antimicrobial effects.

CONCLUSION

The bamboo charcoal extract at all concentrations (12.5%, 25%, 37.5%, 50%, 62.5%) showed no inhibitory effect and was ineffective in inhibiting the growth of *Streptococcus mutans*. Further testing is needed to explore the potential of bamboo charcoal in oral health.

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