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EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF ETHANOLIC GUAVA LEAF (*PSIDIUM GUAJAVA* L.) EXTRACT AGAINST *KLEBSIELLA PNEUMONIAE* AND ITS APPLICATION IN ANTI-HALITOSIS MOUTHWASH FORMULATION

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ABSTRACT

Background: Halitosis is a common oral health problem that can significantly reduce quality of life, primarily due to the activity of bacteria producing volatile sulfur compounds (VSCs). One opportunistic bacterium involved in this condition is *Klebsiella pneumoniae*, which can colonize the oral cavity and produce unpleasant odors. The long-term use of synthetic antiseptic mouthwashes may cause adverse effects and contribute to bacterial resistance; therefore, natural-based alternatives are needed. Guava leaves (*Psidium guajava* L.) are known to contain bioactive compounds such as essential oils, tannins, flavonoids, and saponins, which have potential antibacterial properties.
Objective: This study aims to evaluate the antibacterial activity of ethanolic guava leaf extract (*Psidium guajava* L.) against *Klebsiella pneumoniae* as a potential natural ingredient for anti-halitosis mouthwash formulation.
Methods: Guava leaves were extracted using ethanol as the solvent. The antibacterial activity of the ethanolic guava leaf extract and its mouthwash formulation was evaluated *in vitro* against *Klebsiella pneumoniae* using the agar diffusion method at various extract concentrations.
Results: The results showed that neither the ethanolic guava leaf extract nor its mouthwash formulation exhibited antibacterial activity against *Klebsiella pneumoniae*. This was indicated by the absence of inhibition zones at all tested extract concentrations.
Conclusion: Based on these findings, it can be concluded that the ethanolic extract of guava leaves is not effective as an antibacterial agent against *Klebsiella pneumoniae* and cannot yet be recommended as an active ingredient in mouthwash formulations for halitosis management without further optimization.
Keywords: Halitosis; *Klebsiella pneumoniae*; guava leaves; *Psidium guajava*; antibacterial activity; mouthwash

INTRODUCTION

Halitosis is commonly referred to as oral malodor, is one of the most prevalent oral health problems and significantly affects individuals' quality of life . This condition may originate not only from the oral cavity (intra-oral) but also from extra-oral sources such as the nasal cavity, pharynx, sinuses, and respiratory tract ([1]). Halitosis is primarily caused by volatile sulfur compounds (VSCs), including hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, which are produced through protein degradation by anaerobic microorganisms. In addition to physical discomfort, halitosis has substantial psychosocial consequences, including decreased self-confidence, social withdrawal, anxiety, and cultural stigma, particularly in several Asian societies where it is often perceived as an indicator of overall health status [(2).] The prevalence of halitosis remains high, reaching 25.9% in Indonesia and approximately 25% among the global adult population. Approximately 80–90% of halitosis cases originate from the oral cavity, while the remaining cases are attributed to extra-oral factors [(3)]. Major risk factors include poor oral hygiene, accumulation of tongue coating on the posterior dorsum,

gingivitis, periodontitis, and the proliferation of anaerobic bacteria within oral biofilms. The posterior surface of the tongue, characterized by its rough morphology and anaerobic environment, serves as a primary reservoir for bacteria responsible for VSC production [(4)(5)]. Halitosis is mainly caused by anaerobic bacteria that metabolize sulfur-containing amino acids into malodorous compounds. Including *Actinomyces spp.*, *Fusobacterium nucleatum*, *Streptococcus mutans*, *Staphylococcus aureus*, *Solobacterium moorei* and *Klebsiella pneumoniae.* These microorganisms form complex biofilm consortia on the dorsal surface of the tongue, where anaerobic conditions promote synergistic VSC production [(6)(7).] Furthermore, *Klebsiella pneumoniae*, a gram-negative opportunistic bacterium belonging to the *Enterobacteriaceae* family, may colonize the oral cavity as a result of poor oral hygiene or gastroesophageal reflux. This bacterium possesses a thick polysaccharide capsule that enhances mucosal adhesion, antibiotic resistance, and the production of proteolytic enzymes, leading to ammonia and VSC formation, chronic inflammation, and persistent oral malodor [(5,6).] Conventional management of halitosis generally relies on antiseptic mouthwashes such as chlorhexidine, cetylpyridinium chloride, and essential oil–based formulations. Although these agents are effective in inhibiting oral biofilm formation, long-term use is associated with adverse effects, including mucosal irritation, tooth discoloration, unpleasant taste, disruption of oral microbiota balance, and an increased risk of bacterial resistance. These limitations have encouraged the exploration of safer and more sustainable natural alternatives [(8)(4)]. Several medicinal plants have demonstrated potential as anti-halitosis agents, including betel leaf, basil leaf, bay leaf, and apple peel, which are rich in phenolic compounds and essential oils exhibiting antimicrobial activity against oral pathogens ([9)]. Among these, guava leaves (*Psidium guajava* L.) are particularly notable due to their rich phytochemical composition, including essential oils, tannins, and flavonoids such as quercetin and kaempferol, which possess antibacterial, anti-inflammatory, and antioxidant properties [(10)]. Previous studies have reported the inhibitory effects of guava leaf extracts against pathogenic bacteria, including *Escherichia coli* and *Vibrio cholerae*, through growth inhibition and disruption of cellular functions [(11).] However, studies specifically investigating the antibacterial activity of ethanolic guava leaf extract against *Klebsiella pneumoniae* in the context of halitosis remain limited. Therefore, this study aims to evaluate the antibacterial activity of various concentrations of ethanolic guava leaf extract (*Psidium guajava* L.) against *Klebsiella pneumoniae* as a potential herbal candidate for anti-halitosis mouthwash formulation, with the ultimate goal of reducing reliance on synthetic chemical antiseptics.
cause [12)].

MATERIALS AND METHODS

The study to be conducted is a true experimental study using a post-test only control group design. This design involves three experimental groups: a treatment group, a negative control group, and a positive control group. The treatment group is further divided into four subgroups to obtain different concentrations of guava leaf extract.This study was approved by the Ethics Cittee of the Faculty of Medicine, Udayana University, with the number 1027/UN14.2.2.VII.14/LT/2025.

Preparation of Guava Leaf Simplicia (*Psidium guajava* L.)

A total of 2 kg of fresh guava leaves were collected for simplicia preparation. Initially, wet sorting was performed to remove dust and to separate the required plant parts. The leaves were then sliced and dried in an oven at 50 °C for 9 hours. After drying, dry sorting was conducted, followed by grinding the leaves using a blender to obtain guava leaf simplicia powder. The powder was sieved and stored in a clean, dry, and tightly closed container until further use.

Preparation of Ethanolic Guava Leaf Extract (*Psidium guajava* L.)

Guava leaf extract was prepared using the maceration method. A total of 647 g of dried guava leaf powder was placed into a container and immersed in 3 L of 96% ethanol. The mixture was stirred occasionally during the initial stage and then allowed to stand for 18 hours. The macerate was subsequently separated by filtration. The collected filtrate was concentrated using a rotary evaporator at a temperature of 40–50 °C to obtain a viscous ethanolic extract.

Phytochemical Screening

Qualitative phytochemical screening was conducted to identify the presence of bioactive compounds in the guava leaf extract and the formulated mouthwash preparation. The screening focused on the detection of flavonoids, tannins, and saponins using standard qualitative methods.

Flavonoid identification was performed using the Shinoda test. The guava leaf extract or mouthwash formulation was mixed with magnesium powder, followed by the addition of five drops of concentrated hydrochloric acid. The appearance of a red or orange coloration indicated a positive result, confirming the presence of flavonoid compounds.

T**annin identification** was carried out by adding three drops of ferric chloride ($FeCl_3$) solution to the guava leaf extract or mouthwash formulation. The formation of a dark blue or greenish-black color was considered a positive result, indicating the presence of tannins.

Saponin identification was evaluated using the foam test. The guava leaf extract was shaken vigorously with distilled water in a test tube for 15 minutes. The formation of stable foam approximately 1 cm in height that persisted for at least 15 minutes indicated a positive result for saponin compounds.

Preparation of Mouthwash Formulations

Guava leaf extract mouthwash formulations with concentrations of 10%, 20%, 30%, and 40% were prepared the method with minor modifications.

Preparation of Guava Leaf Extract Mouthwash

The mouthwash was prepared by dispersing the guava leaf extract in a mortar, followed by the addition of glycerin and triturating until completely dissolved. Sorbitol and sodium benzoate were then added and mixed thoroughly. A small amount of distilled water was added to facilitate filtration, after which the solution was filtered into a container. Finally, peppermint oil was added, and the volume was adjusted to 20 mL with distilled water.

Preparation of Nutrient Agar

Nutrient Agar (NA) medium was prepared by dissolving 5.6 g of NA powder in 200 mL of distilled water and homogenizing the mixture using a magnetic stirrer for 15 minutes. The medium was sterilized in an autoclave at 121°C for 15 minutes, cooled, poured into test tubes, and allowed to solidify.

Culture of *Klebsiella pneumoniae*

A pure culture of *Klebsiella pneumoniae* was obtained by inoculating one loopful of bacteria onto Nutrient Agar using the streak plate method. The inoculated plates were incubated at 37°C for 24–48 hours.

Antibacterial Activity Assay

The antibacterial activity of ethanolic guava leaf extract against *Klebsiella pneumoniae* was evaluated using the disc diffusion method on Nutrient Agar. Sterile paper discs (6 mm diameter) were impregnated with guava leaf extract mouthwash at concentrations of 10%, 20%, 30%, and 40%. A commercial mouthwash was used as the positive control, while distilled water served as the negative control. The discs were allowed to stand for approximately 1 hour prior to testing.

Sterile NA medium (20 mL) was aseptically poured into Petri dishes and allowed to solidify. Bacterial suspension was evenly spread onto the agar surface using a sterile swab in a zigzag pattern. The impregnated discs were then placed onto the inoculated agar surface. Each concentration was tested in triplicate. The plates were incubated at 37°C for 24–48 hours, and the inhibition zones were measured using a vernier caliper.

RESULT

Results of Guava Leaf Extraction (*Psidium guajava* L.)

The extraction of guava leaves using the maceration method with 96% ethanol produced a thick extract with a greenish-brown color and a characteristic guava leaf aroma. The extract appeared homogeneous and was suitable for subsequent formulation and antibacterial testing.

Phytochemical Screening Results

Phytochemical screening of the ethanolic guava leaf extract showed the presence of flavonoids, tannins, and saponins. In the guava leaf extract mouthwash formulation, the phytochemical test showed a positive reaction for saponins, while flavonoids and tannins did not show significant reactions.

Antibacterial Activity Test Results

Table 1 Antibacterial Activity of Guava Leaf Extract

Variable	**Average Diameter (mm) of Inhibition Zone Against *Klebsiella pneumoniae***			
	I	**II**	**III**	**Avg.**
Positive control	10,75	9,5	7,25	6,8
Negative control	0	0	0	0
Extract conc.10%	0	0	0	0
Extract conc.20%	0	0	0	0
Extract conc.30%	0	0	0	0
Extract conc. 40%	0	0	0	0

The antibacterial activity test of the ethanolic guava leaf extract against *Klebsiella pneumoniae* using the disc diffusion method showed that no inhibition zones were formed at all tested concentrations (10%, 20%, 30%, and 40%). This indicates that the guava leaf extract at these concentrations did not inhibit the growth of *Klebsiella pneumoniae*. The positive control using a commercial mouthwash produced a clear inhibition zone, while the negative control using distilled water showed no inhibition zone.

Table 2 Antibacterial Activity of Guava Leaf Extract Mouthwash

Variable	**Average Diameter (mm) of Inhibition Zone Against *Klebsiella pneumoniae***			
	I	**II**	**III**	**Avg.**
Positive control	0	8	11,25	6,4
Negative control	0	0	0	0
Extract conc. 10%	0	0	0	0
Extract conc. 20%	0	0	0	0
Extract conc. 30%	0	0	0	0
Extract conc. 40%	0	0	0	0

The antibacterial activity test of the guava leaf extract mouthwash formulation against *Klebsiella pneumoniae* showed that no inhibition zones were observed for all formulations, namely F1 (10%), F2 (20%), F3 (30%), and F4 (40%). These results were consistent across three replicates for each concentration. The positive control demonstrated the formation of an inhibition zone, whereas the negative control showed no inhibition zone.

DISSCUSION

This study evaluated the antibacterial activity of ethanolic guava leaf (*Psidium guajava* L.) extract and its mouthwash formulations against *Klebsiella pneumoniae* using the disc diffusion method. The results demonstrated that neither the crude extract nor the mouthwash formulations at concentrations of 10%, 20%, 30%, and 40% produced inhibition zones against *K. pneumoniae*. In contrast, the positive control exhibited clear antibacterial activity, while the negative control showed no inhibitory effect, confirming the validity of the experimental procedure. Phytochemical screening indicated that the ethanolic guava leaf extract contained flavonoids, tannins, and saponins, which are widely recognized for their antibacterial properties [(1).] However, in the mouthwash formulations, only saponins were detected, whereas flavonoids and tannins were not clearly observed. This finding suggests that the formulation process may have affected the stability or availability of certain bioactive compounds [(13).] The reduction or loss of these constituents may have contributed to the absence of antibacterial activity in the formulated mouthwash. The lack of antibacterial efficacy against *K. pneumoniae* can be attributed to the inherent characteristics of this bacterium. *Klebsiella pneumoniae* is a Gram-negative organism with a complex outer membrane composed of lipopolysaccharides and a thick polysaccharide capsule, both of which serve as effective barriers against antimicrobial agents [(14).] These structural features may limit the penetration of phytochemical compounds, particularly polar constituents commonly found in plant extracts, thereby reducing their antibacterial effectiveness [(15).] Moreover, the disc diffusion method depends on the ability of test substances to diffuse through the agar medium [(16).] The relatively high viscosity of the extract and mouthwash formulations, along with the molecular size of the phytochemical compounds, may have restricted diffusion, resulting in the absence of observable inhibition zones [(17)]. Previous studies have reported antibacterial activity of guava leaf extracts against microorganisms such as *Escherichia coli*, *Vibrio cholerae*, and *Bacillus cereus*; however, differences in cell wall structure and susceptibility among bacterial species may account for the inconsistent results observed against *K. pneumoniae* [(18).] The absence of antibacterial activity in the mouthwash formulations also highlights the importance of formulation

optimization. Interactions between the guava leaf extract and excipients, including glycerin, sorbitol, and sodium benzoate, may have reduced the bioavailability of active compounds (1[9]. Additionally, the formulations did not include penetration enhancers or membrane-disrupting agents that could facilitate the entry of active compounds into Gram-negative bacterial cells [13].

In summary, although guava leaves contain phytochemical constituents with documented antibacterial potential, the ethanolic extract and its mouthwash formulations at the tested concentrations were not effective against *Klebsiella pneumoniae* under the conditions of this study. These findings emphasize the need for further research to optimize extraction techniques, enhance formulation strategies, and explore alternative delivery systems to improve the antibacterial efficacy of guava leaf–based products, particularly for applications targeting oral pathogens associated with halitosis [20].

CONCLUSION AND SUGGESTION

This study concludes that the ethanolic guava leaf (*Psidium guajava* L.) extract formulated as a mouthwash at concentrations of 10–40% was not effective in inhibiting the growth of *Klebsiella pneumoniae*, as no inhibition zones were observed. In contrast, the chlorhexidine mouthwash used as a positive control showed clear antibacterial activity. Further studies are recommended to evaluate higher extract concentrations, apply more sensitive antibacterial methods such as MIC testing, and optimize the formulation to enhance antibacterial efficacy against *Klebsiella pneumoniae*.

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