

Evaluation of the Anti-microbial Properties of Kelulut-derived Lozenges on Oral Pathogens

Anisah Jamaluddin*, Sukirah Abdul Rahman, Azlina Mohd Danial, Mohd Suhaimi Alias, Nur Yuhasliza Abd. Rashid, Mohd.'Azzammil Mohd Asri, Ainur Zunira Md. Saad, and Norman Isman

Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Kelulut honey is documented to exhibit numerous pharmacological advantages, including antibacterial, antioxidant, anti-inflammatory, and anti-apoptotic properties. This study evaluated a newly formulated Kelulut -based lozenge for its antimicrobial efficacy against five oral bacteria: *Streptococcus mutans*, *Streptococcus sobrinus*, *Staphylococcus aureus*, *Actinomyces viscosus*, and *Escherichia coli*, using the agar well diffusion assay method. The findings indicated that the Kelulut-based lozenges exhibited antimicrobial efficacy against all tested bacteria, with the exception of *A. viscosus* and *S. aureus*. The zones of inhibition ranged from 23.00 to 31.5 mm, following this strength sequence of *S. mutans* > *E. coli* > *S. sobrinus*. The results indicated that the Kelulut-based lozenges possess antimicrobial properties against the studied oral pathogens, warranting additional investigation to assess their potential as an alternate product for combating oral pathogens.

Keywords: Antimicrobial, kelulut, lozenges, oral pathogen, stingless bee

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E-mail addresses:

anisj@mardi.gov.my (Anisah Jamaluddin)

sukirah@mardi.gov.my (Sukirah Abdul Rahman)

azlinamd@mardi.gov.my (Azlina Mohd Danial)

suhaimi@mardi.gov.my (Mohd Suhaimi Alias)

yuhass@mardi.gov.my (Nur Yuhasliza Abd. Rashid)

mohdazzm@mardi.gov.my (Mohd.'Azzammil Mohd Asri)

azzuems@mardi.gov.my (Ainur Zunira Md. Saad)

isnorman@mardi.gov.my (Norman Isman)

* Corresponding author

INTRODUCTION

The human oral cavity has more than 700 bacterial species, some of which are pathogenic and may lead to gastrointestinal disorders and tooth loss (Bumm et al., 2021). Oral pathogens also cause dental pulp disease and fungal infections (Santosh et al., 2021) and may lead to both oral and systemic disorders (Bui et al., 2019). Therefore, agents that may specifically suppress oral bacteria are essential to avert disorders induced by oral pathogens.

Oral care is considered a crucial preventive measure for sustaining oral health and, by extension, the overall well-being of the human body (Dudek et al., 2023). Oral health may be preserved by the use of numerous items, such as gels, drinks, or lozenges. Lozenges are flavoured solid dosage forms often used to alleviate local infections and associated symptoms in the oral or oropharyngeal regions. They are extensively used as a support in the treatment of upper respiratory tract infections or as a dietary supplement. Lozenges composed of natural substances are increasingly favoured owing to the diverse pharmacological properties of the molecules within their composition.

In recent years, products produced from natural resources have been the focus of research due to the declining effectiveness of current antibiotics and the alarming rise of increasingly aggressive and resistant microorganisms. This situation has required the scientific community to explore medicinal alternatives derived from natural sources.

Stingless bee honey or famously known as Kelulut honey is known to have high nutritional and therapeutic value. The antimicrobial activity of Kelulut honey, besides helping to preserve the honey itself or some foods, has been useful for wound and burn care, skin, eye, and mucosal infections such as throat diseases and gastrointestinal infections in humans (Cabezas-Mera et al., 2024). The microbiota associated with probiotics and the various combinations of chemicals, including phenols, flavonoids, terpenes, and alkaloids, in stingless bee honey enhance its distinctive biological properties (Machado et al., 2023).

In this context, it may be beneficial to explore the efficacy of Kelulut honey in controlling oral pathogens. Thus, in this study, we examined the antimicrobial activity of a newly developed Kelulut-based lozenge against five oral bacteria: *S. mutans*, *S. sobrinus*, *S. aureus*, *A. viscosus*, and *E. coli*. This preliminary study involves the development of Kelulut honey lozenges, and the evaluation of anti-microbial potential using the agar well diffusion assay.

MATERIALS AND METHODS

Preparation of Kelulut-based Lozenges

The Kelulut honey used in this study was obtained from a local Kelulut farmer in Ketereh, Kelantan. The composition of the Kelulut lozenges comprises sucrose (35%), glucose syrup (33%), Kelulut honey (30%), and water (2%). The mixture of sugar, glucose syrup, and water was heated to 150°C. Subsequently, the Kelulut honey was incorporated into the mixture and heated to 140°C prior to slight cooling, followed by rapid stirring to eliminate bubbles, and poured into moulds, where it was allowed to solidify. For the antimicrobial experiment, each lozenge was dissolved in phosphate-buffered saline (pH 7) at a ratio of 1:1. The lozenge solution was then filtered through a 0.22 µm syringe to ensure sterility before further use.

Preparation of Test Microorganisms

The tested microorganisms for antimicrobial activity were *S. mutans*, *S.sobrinus*, *S. aureus*, *A. viscosus*, and *E. coli*. The bacterial inoculum was maintained and subcultured on Mueller-Hinton agar (MHA) and incubated at 37°C for 24 h. For the antimicrobial experiment, bacterial inocula were produced from overnight cultures.

Antimicrobial Assay—Agar Well-diffusion Assay

The *in vitro* antimicrobial efficacy of Kelulut-based lozenges was assessed using the agar well-diffusion method. The surface of the Muller-Hinton agar plate was inoculated by evenly distributing a volume of the tested microbial inoculum throughout the whole agar surface using a sterile cotton swab. A circular hole is aseptically created in the agar using a sterile cork borer, into which about 100 µL of Kelulut lozenges solution is added. The agar plates were incubated at 37°C to facilitate the diffusion of the Kelulut lozenges solution into the agar medium. After 16 hours, the plates were examined for the zone of inhibition, and the diameters of these zones were measured in millimeters. A clear inhibition zone around the well was the indication of the presence antimicrobial activity and the average was evaluated based on classification by the Clinical and Laboratory Standards Institute (2020).

RESULTS AND DISCUSSION

An inhibition zone or ring that forms around the well after the incubation indicates antimicrobial activity possessed by the studied Kelulut lozenges solution. The observed antimicrobial activity of the lozenge solution against tested oral pathogens is presented in Table 1. The Kelulut lozenges exhibited the greatest inhibitory effects against *Streptococcus mutans* measuring 31.50 ± 0.71 mm (Figure 1A). The inhibitory effect against *S. mutans*, a Gram-positive, facultative anaerobic microorganism is particularly noteworthy, given its pivotal role in the initiation of dental caries which has been a challenge for decades (Ravi et al., 2017).

The Kelulut lozenges also demonstrated considerable inhibitory effects against *E. coli* and *S. sobrinus* with inhibition zones measuring 26.50 ± 0.71 mm and 23.00 ±

Table 1
Antimicrobial susceptibility of Kelulut honey-based lozenges

Test microorganism	Antibacterial activity (zone of inhibition in mm)
<i>Streptococcus mutans</i>	31.50 ± 0.71
<i>Escherichia coli</i>	26.50 ± 0.71
<i>Streptococcus sobrinus</i>	23.00 ± 0.00
<i>Actinomyces viscosus</i>	14.00 ± 0.00
<i>Staphylococcus aureus</i>	-

0.00, respectively (Figures 1B and 1C). Although *E.coli* is frequently linked to intestinal infections, it can also be an oral pathogen, although this occurs less frequently than other bacteria such as *Streptococcus*. *S. sobrinus* is another member of the *Streptococcus* genus that is one of the primary causal pathogens of early dental caries. This is due to their capacity to generate insoluble glucan and fructan and to adhere to the tooth surface (Lee & Kim, 2014). *Streptococci* are also recognized for their ability to aggregate and generate oral biofilms (Jeong et al., 2018). Therefore, inhibiting biofilm formation and decreasing the size of cariogenic bacterial populations are essential approaches to the prevention of oral diseases (Lin et al., 2015). Although Kelulut lozenges exhibited significant inhibition against these pathogens, *A. viscosus* and *S. aureus* exhibited resistance to the lozenges (Figure 1D and 1E).

The observed differential antimicrobial efficacy is likely the result of intricate interactions between the bioactive compounds in the Kelulut honey and the intrinsic resistance mechanisms of each bacterial species (Al-kafaween et al., 2023). As described by Aspar et al. (2020), it is possible that the variation in the antimicrobial efficacy of Kelulut honey against different bacteria is due to its distinctive composition of peroxide and non-peroxide activities, which interact differently with different bacterial strains, thereby affecting their susceptibility to the antibacterial properties of honey. In addition, the mixtures of numerous compounds such as

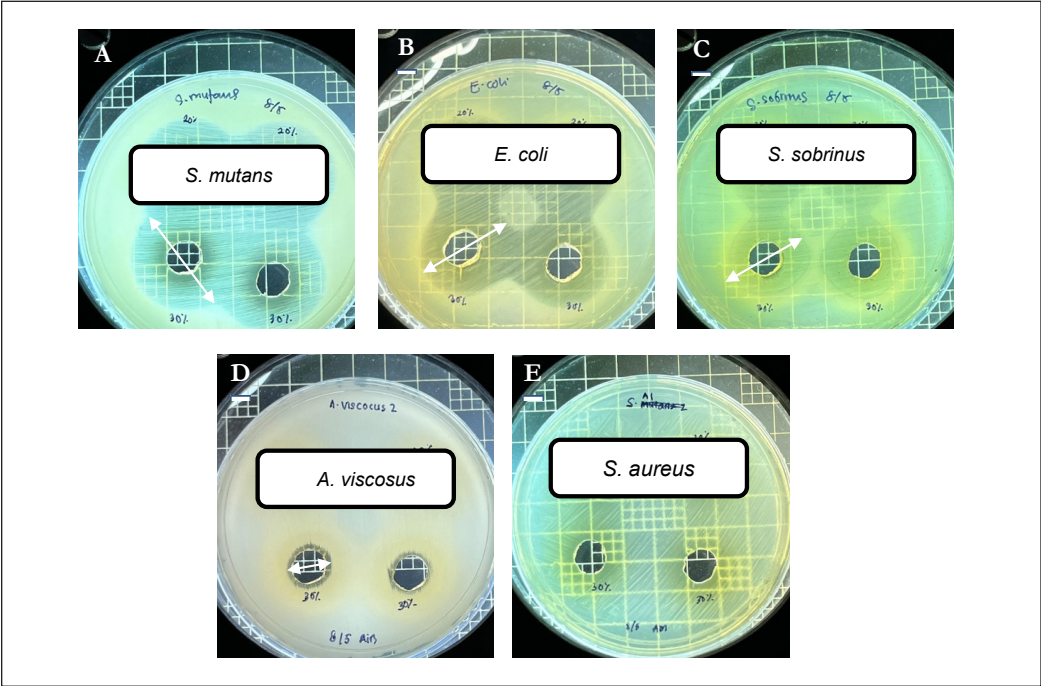


Figure 1. Antimicrobial susceptibility of Kelulut-based lozenges against *Streptococcus mutans*, *Streptococcus sobrinus*, *Staphylococcus aureus*, *Actinomyces viscosus*, and *Escherichia coli*

phenols, flavonoids, terpenes, and alkaloids within Kelulut honey contribute to their unique antimicrobial properties (Machado et al., 2023).

CONCLUSION

According to these findings, Kelulut-based lozenges possess antimicrobial properties against *S. mutans*, *E. coli*, and *S. sobrinus*, with the exception of *A. viscosus* and *S. aureus*, as evidenced by the inhibition zone's manifestation in the agar well diffusion assay. Nevertheless, additional assessment is necessary to ascertain the Kelulut lozenges' potential as a supplement or alternative for the management of oral pathogens.

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