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The roles of acidity, peroxide and non-peroxide compounds in antibacterial properties of Malaysian Kelulut, Tualang and Acacia honey

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ABSTRACT

Aims: In this study, three putative factors that commonly contribute to antibacterial properties in honey were determined, namely acidity (pH level), peroxide compounds and non-peroxide compounds.
Methodology and results: Honey samples were prepared based on the known factors of acidity, peroxide compounds, and non-peroxide compounds to identify factors that contribute to the antibacterial properties of the honey based on agar diffusion assay. Liquid chromatography quadropole time-of-flight mass spectrometry was employed to detect and quantify the presence of acidic, peroxide, and non-peroxide compounds in the honey samples. Acidity and non-peroxide compounds were identified as the significant factors contributing to the antibacterial properties of Kelulut, Tualang and Acacia honey. No peroxide compound was detected in this study across all honey samples. In Kelulut, the presence of the additional compounds (reptoside, platycogenic acid and kauranoic acid) may explain its higher antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* as compared to Tualang and Acacia honey based on the inhibition zones on the agar plates.
Conclusion, significance and impact of study: The presence of multiple antibacterial factors in honey is notably important as it gives an advantage of using honey compared to antibiotics in preventing the growth of a wide range of bacterial species with multiple modes of action.

Keywords: Acidity, antibacterial factors, Malaysian honey, non-peroxide, peroxide

INTRODUCTION

Honey is one of the natural resources used for medicinal purposes due to its antibacterial properties (Almasaudi, 2021). Honey has been proven to possess bacteriostatic and bactericidal properties useful for controlling bacterial infection (Ng *et al.*, 2020), including antibiotic-resistant strains (Brudzynski, 2021). Interestingly, limited studies have reported on the resistance of bacteria towards honey to date (Combarros-fuertes *et al.*, 2020). The presence of potent and irresistible antibacterial properties in honey can be understood by identifying the factors that contribute to its antibacterial properties.

The presence of antibacterial properties of honey is mainly to the osmotic effect (Mandal and Mandal, 2011), acidity (Bogdanov, 1997) and the existence of peroxide and non-peroxide compounds (Almasaudi, 2021). The osmotic effect of honey is produced by strong interactions between sugar and water molecules to reduce the amount of water available to microorganisms (Mandal and Mandal, 2011). As for acidity, it is due to enzymatic action to produce gluconic acid during the ripening of nectar, which creates an acidic environment in honey (Molan, 1992). The acidic pH of honey is generally measured between 3.2 and 5.4, which opposes bacterial growth at an optimum pH between 7.2 and 7.4 (Almasaudi, 2021). Apart from the osmotic effect and acidity, the presence of peroxide and non-peroxide compounds in honey were also identified as among the dominant factors responsible for honey's antibacterial properties (Irish *et al.*, 2011; Kwakman and Zaat, 2012). Peroxide compounds, usually represented by hydrogen peroxide (H_2O_2), cause an increase in oxidative stress that is beneficial to control bacterial colonisation in wound areas (Brudzynski *et al.*, 2011; Combarros-fuertes *et al.*, 2020). The presence of non-peroxide compounds is considered unique since

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