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A review on antibacterial properties of Malaysian kelulut, tualang and acacia honey to prevent wound infectious bacteria

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ABSTRACT

This paper provides a comprehensive review of the antibacterial properties of three different types of Malaysian honey, namely kelulut, tualang and acacia, in preventing wound infecting bacteria. The antibacterial activity of these honey is mainly attributed to the physicochemical and phytochemical, which have been shown to be effective against a broad range of bacterial pathogens. This review discusses the effectiveness of honey in inhibiting the growth of various wound-infecting bacteria, factors that contributed to the antibacterial properties of the honey, mechanisms of action of honey in inhibiting bacterial growth and their potential for future use in clinical practice. The findings of this review suggest that Malaysian honey has the potential to be used as an alternative and complementary therapy to conventional antibiotics in the prevention and treatment of wound infections.

Keywords: Antibacterial properties, infectious bacteria, Malaysian honey, wound

INTRODUCTION

Honey is a sweet, viscous substance made by honey bees and stingless bees from flower nectar and plant saps (honeydew). Since ancient times, honey has traditionally been used as a medicine or tonic rather than a staple diet due to its biological properties. Among the biological properties, honey is known for its antibacterial properties (Islam *et al.*, 2020; Almasaudi, 2021). Many studies reported the antibacterial properties of honey harvested worldwide against various infectious bacteria, as it possesses the capacity to be both bacteriostatic and bactericidal (Girma *et al.*, 2019; Al-kafaween *et al.*, 2020). The bacteriostatic effect is the ability to inhibit at least 90% of bacterial growth (Brudzynski and Sjaarda, 2014) and the bactericidal effect is the ability to eliminate the growth of bacteria (Shehu *et al.*, 2015).

Bacterial infection is the most common contamination of wound that delays the rate and reduce the quality of the healing process by causing failure of grafts and flaps formation as a repair mechanism (Simões *et al.*, 2018). As soon as the skin was impaired, typical microorganisms of the normal skin flora gained access to underlying tissues, which offers a humid, warm, and nutrient-rich environment for their development (Bowler *et al.*, 2001; Serra *et al.*, 2015). If left untreated, this results in an infected wound and will eventually delay the healing process. When healing is delayed, the normal microbiota of wounds become more aggressive microbial types that proliferate and colonise into deeper skin layers affecting the tissues (Negut *et al.*, 2018; Simões *et al.*, 2018). The delay and untreated condition with diverse types of bacteria and other pathogens are key to the emergence of various immune-related, inflammatory, and certain cancerous conditions. Due to its antibacterial properties, honey is an alternative that can be used to initially prevent bacterial infection and boost the process of wound healing. Based on its low pH, low water activity and presence of antibacterial compounds, i.e., peroxide and non-peroxide, honey provides a protective barrier which simultaneously treats and prevents microbial from infecting wound (Zakaria, 2015). Honey has been proposed as antibacterial agent to treat bacterial infection. The advantages of using honey were due to its naturally available, non-toxic and most important, effective against resistant strains without reproducing tolerable resistant strain (Combarros-Fuertes *et al.*, 2020; Ng *et al.*, 2020).

In recent years, several preliminary studies have been conducted to evaluate the antibacterial properties of Malaysian honey against bacteria that are associated with wounds (Mohd-Aspar *et al.*, 2020; Gopal *et al.*, 2021). The studies considering the honey actively harvested in

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Malaysia, such as tualang (Mohd *et al.*, 2020), kelulut (Omar *et al.*, 2019), gelam (A-Rahaman *et al.*, 2013), pineapple (Kek *et al.*, 2014), coconut (Samat *et al.*, 2014), royal honey (Hamid *et al.*, 2018) and acacia (Mohd-Aspar *et al.*, 2019). However, due to low production and high-temperature sensitivity, honey such as gelam, pineapple, coconut and royal honey were limited in their resources. However, it is not the same case for tualang, kelulut and acacia honey. Therefore, this review aims to report on the antibacterial properties of widespread types of honey harvested in Malaysia known as tualang, kelulut and acacia honey against wound-infecting bacteria. The discussion to uncover the antibacterial efficacy of the honey, factors that contribute on presence of antibacterial properties, mechanism of antibacterial action and potential as an alternative in clinical applications.

Malaysian tualang, kelulut and acacia honey

Bees collect honey and transform it through the combination with specific substances of their own; then deposit, dehydrate, store, and leave it in honeycombs to ripen and mature. Various kinds of honey are available worldwide. The variety of honey is determined mainly based on the types of bees that produce the honey and the sources of pollen and nectar to make the honey (Amin *et al.*, 2018). Each kind of honey is identified with identical physicochemical properties such as colour, pH, and taste. The variation in physicochemical properties of honey is due to the source of nectar collected, season, environmental factors and species of bee that produced the honey (Boukraâ, 2013).

Tualang

Tualang honey is a multiflora honey produced by *Apis dorsata*, which is also known as Asian rock bees or the giant honeybee that collect pollen and nectar from various wild plants in Malaysia's rainforest jungle (Syazana *et al.*, 2013). The collected pollen and nectar were stored in the hive that is built at the branches of the tualang tree, scientifically known as *Koompassia excelsa*, which is mainly found in the tropical rainforest and can grow to approximately 250 feet in height (Ahmed and Othman, 2013). Other than Malaysia, tualang tree could also be found in other Southeast Asian countries such as Indonesia, Philippines, and Thailand. It is one of the tallest tropical tree species, with the tallest measured was 88 m tall (Soepadmo *et al.*, 1995). Based on the fact that tualang honey was harvested from tualang tree, it was named according to the tree where it was harvested. Tualang honey can be found in a various range of colours and tastes depending on the nectar and pollen collected by the worker bee. Tualang honey can be found in a dark to light amber colour. The taste varies between bitter-sweet and sweet-sour. Usually, tualang honey harvested in Malaysia is dark amber with a slightly bitter-sweet taste (Rao *et al.*, 2016). Tualang honey is commonly consumed as a nutritious food or tonic with medicinal effects in Malaysia (Ismail, 2016). The details on the physicochemical properties of tualang honey are tabulated in Table 1.

Kelulut

Kelulut or stingless bee honey, is produced by stingless bees from the genus of *Trigona*, which are small in size like the fly (Yaacob *et al.*, 2018). Techniques on culturing the stingless bees involved either meliponiculture or wild. Due to its characteristic that does not sting, kelulut honey is usually harvested from meliponiculture with modern moveable comb hives built in farms (Kelly *et al.*, 2014). Meanwhile, the hive for wild kelulut is commonly built at the root and stump of trees. In Malaysia, kelulut are harvested from both meliponiculture and the wild. The colour of this multiflora honey depends mainly on the sources (nectar and pollen) collected by the stingless bees, and in Malaysia, the colour ranges from light to dark amber (Fatima *et al.*, 2018) with an identical sweet-sour taste. The commercial harvesting of kelulut was first introduced in Kelulut Park, MARDI Centre, Serdang, in 2011 and continues to increase (Ismail, 2016). Despite it being commercially recognised, the amount of research conducted on its bioactive properties is still lacking, which demanding further investigation. The details on the physicochemical properties of kelulut honey are tabulated in Table 1.

Acacia

Acacia honey is monofloral honey derived from *Acacia mangium* produced either by *Apis mellifera* or *Apis cerana* (Zainol *et al.*, 2013; Samat *et al.*, 2014). The honey is usually harvested from apiculture with modern moveable comb hives built in farms using wood. In Malaysia, several farms are reserved for *A. mangium* trees meant for apiculture, such as in the states of Johor and Sarawak (A-Rahaman *et al.*, 2013). The taste of acacia honey is sweet and flowery as it is produced from nectar and honeydew from *A. mangium* tree (Chan *et al.*, 2017). The colour of acacia honey is usually found between light and slightly dark amber. Acacia is a well-known honey commonly utilised as a food source in Malaysia. The details of its physicochemical properties are tabulated in Table 1.

Bacteria infecting wound

The bacterial infection is the primary cause of delayed wound healing (Bowler *et al.*, 2001). The infected wound is usually a polymicrobial infection primarily derived from Gram-positive (Serra *et al.*, 2015) and Gram-negative bacteria (Negut *et al.*, 2018). The Gram-positive and Gram-negative bacteria that commonly infect wound include *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The bacteria may infect various kinds of wounds, including those with acute and chronic conditions such as acute foot ulcer (Kateel *et al.*, 2018), skin ulcer (Yang *et al.*, 2017), post-surgical wound (Kasithevar *et al.*, 2017), traumatic wound and diabetic

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Table 1: Physicochemical properties of tualang, kelulut and acacia honey harvested in Malaysia.

	Tualang (Ahmed and Othman, 2013; Moniruzzaman *et al.*, 2013; Mohd-Aspar *et al.*, 2020)	Kelulut (Chan *et al.*, 2017; Lani *et al.*, 2017; Mohd-Aspar *et al.*, 2020)	Acacia (A-Rahaman *et al.*, 2013; Moniruzzaman *et al.*, 2013; Mohd-Aspar *et al.*, 2020)
Appearance	Dark brown	Light to dark amber	Light to slightly dark amber
Moisture content (%)	23.3	21.4-31.6	15.2 - 19.5
pH	3.6-4.0	2.4-4.2	3.3 - 4.3
Total reducing sugar (%)	67.5	55.0-86.0	69.1 - 70.3
Glucose (%)	30.0	8.2-30.9	33.3
Fructose (%)	29.6	31.1-40.2	43.1
Sucrose (%)	0.6	0.3-1.3	1.2
Maltose (%)	7.9	33.7-45.2	1.2
Electrical conductivity (mS/cm)	0.7-1.4	0.5-8.8	1.1
HMF (mg/kg)	46.2	40.0	0.3

Table 2: Bacteria commonly isolated from infected wounds.

Bacteria	Reference
Gram-positive	
Staphyloccoccus aureus	(Kateel *et al.*, 2018; Cheung *et al.*, 2021)
Staphylococcus haemolyticus	(Yang *et al.*, 2017; Eltwisy *et al.*, 2020)
Streptococcus pyogenes	(Abd El-Malek *et al.*, 2017; Fukuta *et al.*, 2022)
Streptococcus agalactiae	(Fukuta *et al.*, 2022)
Enterococcus faecalis	(da Silva *et al.*, 2022)
Gram-negative	
Escherichia coli	(Kateel *et al.*, 2018; Misha *et al.*, 2021)
Pseudomonas aeruginosa	(Prasad *et al.*, 2020)
Salmonella typhimurium	(Yang *et al.*, 2017; Qin *et al.*, 2020)
Staphylococcus epidermidis	(Amato *et al.*, 2018; Byrd *et al.*, 2018)
Bacillus cereus	(Malik-Tabassum *et al.*, 2017; Tusgul *et al.*, 2017)
Acinetobacter baumannii	(Abd El-Malek *et al.*, 2017; Shariati *et al.*, 2021)
Proteus mirabilis	(Armbruster *et al.*, 2018)
Proteus vulgaris	(Drzewiecka, 2016)
Enterobacter clocae	(Davin-Regli *et al.*, 2019)
Enterobacter aerogenes	(Wong *et al.*, 2013; Davin-Regli *et al.*, 2019)
Klebsiella pneumonia	(Hanina *et al.*, 2015; Shariati *et al.*, 2021)

foot ulcer (Abd El-Malek *et al.*, 2017). The bacteria commonly isolated from the infected wound sites are tabulated in Table 2.

The bacteria that infect wounds are generally capable of developing various mechanisms of resistance towards antibiotic treatment, such as forming biofilm (Fiedler *et al.*, 2015) and producing enzymes such as beta-lactamase (Ventola, 2015; Almasaudi, 2018) and catalase (Horn *et al.*, 2018) to eliminate the effectiveness of antibiotic treatments. Other than the ability to develop resistance mechanisms, the wound infectious bacteria also possess the ability to adapt and continue growing in various environmental conditions, including extremely low and high pH (between pH 2.5 to 10) (Food and Drug Administration, 2011; Kim *et al.*, 2018) and temperature (between 4 °C to 46 °C) (Keerthirathne *et al.*, 2016; Dekic *et al.*, 2018;) by rapid adjustment of the lipid membrane composition (Siliakus *et al.*, 2017) and modification of genetic mechanisms (Beales, 2004). These characteristics dictate the flexibility and difficulty of preventing bacteria from continuously infecting wounds.

Antibacterial efficacy of the tualang, kelulut and acacia honey against wound infectious bacteria

A substance's antibacterial property is slowing down the growth or total elimination of bacteria. The compounds or substances that obtain these properties are called antibacterial agents. Antibiotics are antimicrobial substances active against bacteria and are the most important antibacterial agents for preventing bacterial infections (Fukuta *et al.*, 2022). The antibacterial properties of antibacterial agents can be generally classified as bacteriostatic and bactericidal, which is determined based on its effect on microorganisms. The bacteriostatic effect is determined based on the minimum inhibitory concentration (MIC) of agent to inhibit at least 90% of bacterial growth (Brudzynski and Sjaarda, 2014),

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Table 3: The MIC and MBC of Malaysian tualang, kelulut and acacia honey against Gram-positive bacteria associated with wound infection.

	(%)	Tualang (Tan *et al.*, 2009; Zainol *et al.*, 2013; Shehu *et al.*, 2015; Mohd *et al.*, 2020)	Kelulut (Zainol *et al.*, 2013; Tuksitha *et al.*, 2018; Mohd-Aspar *et al.*, 2020)	Acacia (Zainol *et al.*, 2013; Mohd-Aspar *et al.*, 2019)
Staphylococcus aureus	MIC	10-40	10-17.5	20-30
	MBC	15-50	20-30	25->90
Streptococcus pyogenes	MIC	30	10-20	30-40
	MBC	60-90	20	>90
Enterococcus faecalis	MIC	40	20	50
	MBC	>90	50	>90
Staphylococcus hominis	MIC	15	6.25	50
	MBC	30	25	>90
Staphylococcus haemolyticus	MIC	12.5	<5	40
	MBC	50	25	>90
Streptococcus agalactiae	MIC	30	10	40
	MBC	60	20	>90

while the bactericidal effect is determined based on the minimum bactericidal concentration (MBC) of agent to eliminate the growth of bacteria (Shehu *et al.*, 2015).

In Malaysia, kelulut, tualang and acacia honey are popular types of honey that have been studied on their antibacterial properties compared to other available honey (Shehu *et al.*, 2015; Tuksitha *et al.*, 2018). Table 3 and Table 4 tabulate the ranges of MIC and MBC for tualang, kelulut and acacia honey against Gram-positive and Gram-negative bacteria that are associated with wound infection, respectively.

Kelulut honey has been shown to have MIC values ranging from 3.75% to 20% (w/v) against various wound infectious bacterial strains, including *S. aureus* and *E. coli*. In addition, kelulut honey has been found to have MBC values ranging from 10% to 50% (w/v) against these bacterial strains. As for tualang, honey has been found to have MIC values ranging from 5% to 40% (w/v) against various wound infectious bacterial strains. In addition, tualang honey has been shown to have MBC values ranging from 10% to >90% (w/v) against these bacterial strains. Acacia honey has been shown to have MIC values ranging from 5% to 50% (w/v) against various bacterial strains. In addition, acacia honey has been found to have MBC values ranging from 10% to >90% (w/v) against these bacterial strains.

These values of MICs and MBCs suggest that kelulut, tualang and acacia honey effectively inhibit the growth of wound infectious bacteria at relatively low concentrations and can also kill them at higher concentrations. However, the effectiveness of each type of honey may vary depending on the specific bacterial strain being tested, as well as other factors such as the honey's geographical origin and processing methods.

Factor contributed to antibacterial properties of honey

The antibacterial properties of honey have been known to exist due to certain factors that can be explained by physicochemical and phytochemical properties. Here, the contribution of physicochemical properties and phytochemical properties in the antibacterial properties of kelulut, tualang and acacia honey are discussed.

Physicochemical properties

The physicochemical properties are a response related to both physical and chemical elements. The physicochemical properties of honey can have a significant impact on its antibacterial properties. Water activity, sugar

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Table 4: The MIC and MBC of Malaysian tualang, kelulut and acacia honey against Gram-negative bacteria associate with wound infection.

	(%)	Tualang (Tan *et al.*, 2009; Zainol *et al.*, 2013; Shehu *et al.*, 2015; Mohd *et al.*, 2020)	Kelulut (Zainol *et al.*, 2013; Tuksitha *et al.*, 2018; Mohd-Aspar *et al.*, 2020)	Acacia (Zainol *et al.*, 2013; Mohd-Aspar *et al.*, 2019)
Pseudomonas aeruginosa	MIC	5-20	3.75-20	5-30
	MBC	10-40	10-20	10-50
Escherichia coli	MIC	15-25	7.5-20	25-40
	MBC	25->90	40-50	50->90
Klebsiella pneumonia	MIC	30-40	10-12.5	30
	MBC	80->90	30	>90
Salmonella species	MIC	20	7.5	40
	MBC	60	25	>90
Acinetobacter baumannii	MIC	15	<5	30
	MBC	60	12.5	>90
Enterobacter clocae	MIC	25	7.5	50
	MBC	>90	20	>90
Enterobacter aerogenes	MIC	30	12.5	40
	MBC	60	25	>90
Proteus mirabilis	MIC	25	7.5	40
	MBC	90->90	25	>90
Ptroteus vulgaris	MIC	15	<5	25
	MBC	30	20	40

content and acidity are among the physicochemical properties affecting the antibacterial properties of kelulut, tualang and acacia honey.

Water activity and sugar content

The osmotic pressure in honey is mainly due to the presence of a high concentration of sugar combined with low water activity (Johnston *et al.*, 2018). In honey, the interaction of sugar (glucose, fructose, sucrose and maltose) with water leaves very few free water molecules available for microorganisms (Mandal and Mandal, 2011). Water requirements of microorganisms are generally described in terms of water activity (α_w). α_w is defined as the ratio of the vapour pressure of water in a material to the vapour pressure of pure water at the same temperature. Many bacteria prefer α_w of 0.99 and most need α_w higher than 0.91 to grow. Higher α_w tends to support more microorganisms due to a sufficiently diluted aqueous environment that helps the growth of microorganisms (Mugnire and Jung, 1985). The growth of bacteria could be inhibited at α_w of 0.94 and below (Molan, 1992). In pure-distilled water, α_w has the value of precisely one, while α_w of honey was found to be 0.6, which is equivalent to 23% of water content by weight (Bradbear, 2009). This condition is considered too low to support the growth of many bacterial species. At higher dilutions of 30%, the antibacterial properties of honey are due to factors other than osmotic stress (Kwakman and Zaat, 2012). Malaysian tualang, kelulut and acacia honey possess a moisture content of 23.3%, 31.6% and 19.5%, respectively. Tualang and acacia honey are considered as very low to support the growth of bacteria with α_w of 0.6. As for kelulut, higher α_w was observed to indicate the presence of other factors that contribute to its antibacterial properties.

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Acidity

The acidity of honey is primarily due to the content of gluconic acid resulting from enzymatic action during the ripening of the nectar (Molan, 1992). The acidity of honey with pH ranging from 2.4 to 5.4 are inhibitory to bacterial growth (Bogdanov, 1997). In general, pathogens do not grow or grow at a very slow rate at pH levels below 4.6, and the optimum pH for bacterial growth is between 7.2 and 7.4 (Molan, 1992). The minimum pH for growth of several infectious bacteria species, i.e., *E. coli*, *Salmonella* sp., *P. aeruginosa*, *S. aureus* and *S. pyogenes* were 4.4, 4.2, 4.4, 4.0 and 4.5, respectively (Molan, 1992).

A study that evaluated the contribution of low pH of Malaysian tualang, kelulut and acacia honey against bacteria infecting wounds demonstrated that the neutralisation of acidity of honey from 3.3 to 7.0 has significantly reduced the inhibition zone (Omar *et al.*, 2019). The finding was in parallel with another study that evaluated the contribution of low pH to the bactericidal effect of honey from the Netherlands towards clinically isolated bacteria and demonstrated that the neutralisation of acidity of honey from 3.4 to 7.0 and combined with the neutralisation of other bactericidal factors including hydrogen peroxide (H_2O_2), Methylglyoxal (MGO) and bee defensin-1 reduced the bactericidal effect of honey to the same level of a sugar-based artificial honey solution (Kwakman *et al.*, 2010). However, the findings contradict another study on the acidity of honey harvested in Denmark, which demonstrated the low pH of honey in the range of 3.3 to 3.8 did not seem to be a crucial antibacterial factor in honey (Matzen *et al.*, 2018).

The complexity of ingredients presented in different types of honey may affect the antibacterial properties. Although specific observations have highlighted that acidity is not a crucial factor to the antibacterial properties of honey, the elimination of an acidic environment does partially contribute to antibacterial properties in honey for inhibiting infectious bacteria generally (Molan, 1992; Kwakman *et al.*, 2010). As for Malaysian tualang, kelulut and acacia, acidity is among the crucial factors that affect the antibacterial properties of honey against wound infectious bacteria (Mohd-Aspar *et al.*, 2023).

Phytochemical compounds

Phytochemical, also known as phytonutrient, refers to any of the bioactive chemical compounds found in plants that are beneficial to human health. Several compounds with phytochemical properties are discovered in kelulut, tualang and acacia honey, and these are polyphenols compounds (phenolic acids and flavonoids) and H_2O_2. Other unavailable polyphenols in kelulut, tualang and acacia honey, such as methylglyoxal and antimicrobial peptide were also discussed. The summary is tabulated in Table 5.

Polyphenols compounds

Polyphenols or phenolics are among the essential groups of compounds existing in plants that are proven to have antibacterial (Sánchez-Maldonado *et al.*, 2011), anti-inflammatory (Kumar and Pandey, 2013) and antioxidant properties (Ranneh *et al.*, 2018). Polyphenol compounds are present in honey and have been reported to have some chemo-protective effects in humans. Polyphenols found in honey were mostly denoted by phenolic acids and flavonoids.

The phenolic acids are generally divided into two subclasses, i.e., benzoic acids and cinnamic acids, whereas the flavonoids present in honey are categorised into three classes, i.e., flavonols, flavones and flavanones (Amin *et al.*, 2018). Generally, the physical appearance of honey that is darker indicates higher phenolic acids and flavonoid compounds as compared to lighter honey (Sousa *et al.*, 2016; Bakar *et al.*, 2017). The composition of polyphenol compounds in honey varies depending mainly on the floral sources and also external factors such as seasonal, environmental factors and processing (Ranneh *et al.*, 2018). Different compositions of polyphenol compounds are expected in honey harvested from different regions (Johnston *et al.*, 2018). Flavonoids and phenolic acids are among the factors that contributed to the presence of antibacterial properties in Malaysian kelulut (Chan *et al.*, 2017; Tuksitha *et al.*, 2018), tualang (Ranneh *et al.*, 2018) and acacia honey (Salleh *et al.*, 2017).

In a study that evaluated the contribution of the non-peroxide compound to inhibit bacteria growth demonstrated that neutralisation of acidity and H_2O_2 in kelulut, tualang and acacia honey significantly reduced the inhibition zone towards *E. coli* and *S. aureus* (Zainol *et al.*, 2013). The contribution of polyphenols in antibacterial properties of the honey were verified through identification of compounds using LC-QTOF MS that obtained the presence of the flavonoids such as 3',4',7-trihydroxyisoflavanone, 5,7,4′-trihydroxy-8,3′-diprenylflavone and 3′,4′-dimethoxy-isoflavan-7,2′-di-O-β-D-glucoside (Mohd-Aspar *et al.*, 2023).

Hydrogen peroxide

Peroxides are a group of compounds with the structure R-O-O-R in which O-O structure is known as the peroxide group. The most common peroxide is H_2O_2 which is identified as a powerful antibacterial compound identified in most honey (Irish *et al.*, 2011; Mandal and Mandal, 2011; Kwakman and Zaat, 2012) that is beneficial in controlling bacterial colonisation through oxidative stress (Brudzynski *et al.*, 2011; Zainol *et al.*, 2013).

H_2O_2, in its pure form, is a colourless and odourless water-soluble liquid that consists of hydrogen and oxygen components (Urban *et al.*, 2017). It is an unstable compound that decomposes in the presence of catalase (which is found predominantly in the liver) and is also inhibited by excessive heat (temperature of 75 °C and above) and very low water activity (less than 18% water

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Table 5: Presence of compounds that correspond to phytochemical properties in tualang, kelulut and acacia honey.

Compounds	Tualang	Kelulut	Acacia	References
Phenolic acids	Presented	Presented	Presented	(Salleh *et al.*, 2017; Ranneh *et al.*, 2018; Tuksitha *et al.*, 2018; Mohd-Aspar *et al.*, 2023)
Flavonoids	Presented	Presented	Presented	(Zainol *et al.*, 2013; Chan *et al.*, 2017; Ranneh *et al.*, 2018; Tuksitha *et al.*, 2018; Mohd-Aspar *et al.*, 2023)
Hydrogen peroxide	Presented	Presented	Presented	(Ahmed and Othman, 2013; Zainol *et al.*, 2013; Nishio *et al.*, 2016)
Antimicrobial peptides	NA	NA	NA	-
MGO	NA	NA	NA	-

NA: No data available.

content) (Kwakman and Zaat, 2012). H_2O_2 is known for its antimicrobial and cleansing effects when it was first introduced into clinical practice. The antimicrobial properties of H_2O_2 provide broad-spectrum efficacy against Gram-positive and Gram-negative bacteria, viruses, and yeast (Urban *et al.*, 2017). A 3% H_2O_2 solution is a safe and effective irrigation solution for intraoperative wound cleansing and haemostasis (Urban *et al.*, 2017).

In honey, H_2O_2 is produced by the activation of the enzyme glucose oxidase that oxidises glucose. The H_2O_2 concentration produced in honey is very low, typically about 900-fold lower than in a 3% solution, which is commonly used as an antiseptic (Brudzynski *et al.*, 2011). Despite its low level, H_2O_2 is still effective as an antibacterial compound that synergistically reacts with other factors in honey. It has been demonstrated that the low concentration of H_2O_2 in honey was necessary to cause the degradation of bacterial DNA compared to H_2O_2 alone, which required a similar effect to occur at a higher concentration (approximately 2.5mM) (Brudzynski *et al.*, 2011). The enzymatic action of glucose oxidase is inactive in pure honey, and the H_2O_2 level is said to be minimised (White *et al.*, 1963). Upon dilution, the enzyme can be active and start to utilise glucose to produce H_2O_2 when honey is diluted in the range of 30% to 50% and declines rapidly below 30% due to the relatively low affinity of glucose oxidase to react with glucose (Molan, 1992; Kwakman and Zaat, 2012).

In a study on the contribution of peroxide compounds in kelulut, tualang and acacia honey to inhibit the growth of infectious bacteria, the activation of peroxide compounds was observed at a concentration of 40% (w/v). The effect denoted by peroxide compounds was most prominent in kelulut as compared to tualang and acacia honey. Certain types of honey are highly peroxide-dependent, such as Ulmo honey, which possesses very low antibacterial action when treated with catalase (Sherlock *et al.*, 2010), while others are poor or none peroxide-dependent including kelulut, tualang, acacia and manuka honey (Kwakman *et al.*, 2011). Due to complications such as instability and inactivation of H_2O_2, the antibacterial effect of honey does not sustain if it entirely depends on the presence of H_2O_2.

Antimicrobial peptide

The honey bee hypopharyngeal gland secretes antimicrobial peptides for the production of honey. In honeybees, four types of peptides are produced, and these are hemenoptecin, bee defensin-1, apidaecin and the group of abaecin peptides. Each of these peptides has a distinct spectrum of antimicrobial activity, and collectively, these peptides cover all major classes of microorganisms. Among the peptides, bee defensin-1 was identified in honey (Kwakman *et al.*, 2011). Bee defensin-1 has potent antibacterial properties against several bacteria, such as *B. subtilis* and *S. aureus* (Kwakman and Zaat, 2012). However, the presence of antimicrobial peptides in kelulut, tualang and acacia honey has not been systematically investigated and quantitative data on the concentration of this peptide have not yet been established.

Methylglyoxal

Methylglyoxal (MGO) is a chemical compound found in varying amounts in different types of honey. It is produced from dihydroxyacetone (DHA), a simple sugar that is naturally present in nectar. MGO forms naturally by non-enzymatic conversion of dihydroxyacetone (DHA) at a slow rate during storage of the honey (Adams *et al.*, 2009; Kwakman and Zaat, 2012). MGO was revealed as the unique compound that contributes to the presence of antibacterial properties in honey since it is a non-peroxide compound that cannot be destroyed by catalase (Adams *et al.*, 2008). There is a strong correlation between the MGO levels and the antibacterial properties of honey (Adams *et al.*, 2008). It is important to note that the MGO content of honey can vary depending on factors such as the type of flower the bee's collected nectar from, the season and the geographical location where the honey was produced.

MGO has been identified as the dominant antibacterial compound in manuka honey, a well-known honey with high antibacterial properties (Kwakman *et al.*, 2011). Concentrations of MGO in various foods have been reported in the range of 3 mg/kg to 47 mg/kg. As a comparison, manuka contains concentrations of MGO that range from 38 mg/kg to 1541 mg/kg, which is higher by 32-fold (Adams *et al.*, 2008; Stephens *et al.*, 2010).

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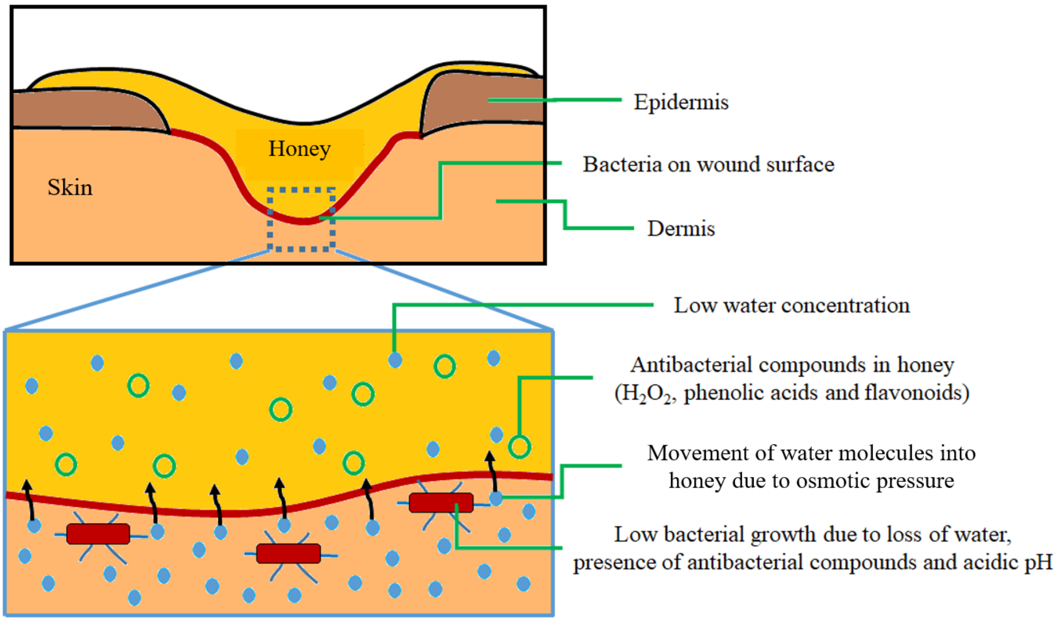

Figure 1: Illustration of the antibacterial action of honey on wound surface to prevent microbial growth.

Other than manuka, MGO is also present in honey harvested from other plant species but in low concentration that does not exceed 174 mg/kg (Stephens *et al.*, 2010). However, similar to antimicrobial peptides, the presence of MGO in kelulut, tualang and acacia honey has not been identified, and to the best of our knowledge, the quantitative data on the concentration of MGO in this honey have not yet been established.

Mechanism of antibacterial action of the Malaysian kelulut, tualang and acacia honey

Based on the various factors that contribute to antibacterial properties, Malaysian kelulut, tualang and acacia honey showed a broad spectrum of antibacterial actions which were found to vary in its effect on different bacterial species. Interestingly, the broad spectrum of antibacterial actions acts simultaneously in inhibiting infectious bacteria to result in the declination of virulence genes' expression (Al-kafaween *et al.*, 2021), prevention of biofilm formation (Al-kafaween *et al.*, 2020) and cell wall disruption or lysis through the formation of spheroplasts (Zakaria, 2015; Nishio *et al.*, 2016). Although limited findings have been reported on the mechanism of antibacterial action, these honeys are expected to have broad antibacterial properties considering the physicochemical and phytochemical properties presented in them (Figure 1). The broad antibacterial actions presented in this honey are a major advantage as compared to antibiotics that are dependent on a single factor for its antibacterial action, which is easily resisted by bacteria, i.e., beta-lactam in penicillin (Peacock and Paterson, 2015).

In summary, the antibacterial mechanism of action of kelulut, tualang and acacia honey involves multifactorial effects that require further investigation. It is rather precise to generalising the action due to the synergic effects of osmotic pressure, acidity, peroxide and non-peroxide compounds.

Potential use of the honey in preventing wound from infected by bacteria

Wound infectious are a major concern in healthcare settings due to their potential to cause severe complications, increase hospital stays and incur high treatment costs. Antibiotics are commonly used to prevent and treat wound infections, but their overuse has led to the emergence of antibiotic-resistant bacterial strains. As such, there is a growing interest in the use of natural products, such as honey, as an alternative and complementary therapy to conventional antibiotics. Among the different types of honey available, kelulut, tualang and acacia honey are known to have potent antibacterial properties and have been studied extensively for their potential use in preventing wound infections.

The use of kelulut, tualang and acacia honey in preventing wound infections holds great promise, particularly in the era of antibiotic resistance. These honeys are used to be effective against a broad range of bacteria and have the potential to be used as alternative and complementary therapies to conventional antibiotics. In addition, they have other beneficial properties, such as anti-inflammatory (Ranneh *et al.*, 2021) and wound healing (Devasvaran and Yong, 2016) activities that may enhance their effectiveness in preventing and treating wound infections.

The effort to propose Malaysian honey as a topical preparation was initiated by several studies (Khoo *et al.*,

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2010; Mohd Nasir *et al.*, 2010). In a clinical study conducted by Mohd Nasir (2010) the effectiveness of employing tualang honey in burn wound dressing was evaluated. A standard clinical plain dressing was soaked with tualang honey before being applied on selected patients with partial thickness burns for 3, 6 and 9 days. The results showed that tualang honey is beneficial as a dressing, having both bactericidal and bacteriostatic effects. In addition, the dressing was easier to apply and is less sticky compared to Manuka honey. In another study by Khoo (2010), burn wounds on 36 female Sprague Dawley rats were dressed with tualang honey, hydrofibre and hydrofibre silver, respectively, before obtaining swab samples on day 3, 6, 9, 12, 15, 18 and 21 for quantitative and semi-quantitative microbiological analyses. The study found that tualang honey-treated rats demonstrated better results with regard to its control of *P. aeruginosa* and its wound contraction effects on full-thickness burn wounds *in vivo*. Although initiative studies have been conducted, further investigation are needed to determine the optimal doses and suitable application methods of Malaysian honey in clinical practice.

CONCLUSION

In conclusion, this review provides an overview of the antibacterial properties of kelulut, tualang and acacia honey in preventing the growth of wound infection bacteria. The antibacterial properties of these honey include both bacteriostatic and bactericidal effects which are shown to be effective against a broad range of bacteria for both Gram-positive and Gram-negative organisms. The potent antibacterial properties owned by kelulut, tualang and acacia honey were attributed to the presence of physicochemical and phytochemicals that dominated by acidic pH and the existence of polyphenols and hydrogen peroxide. The potential use of Malaysian kelulut, tualang, and acacia honey in preventing wound infections represents an exciting area of research with significant implications for healthcare. As the challenges surrounding the efficacy of antibiotics against resistant bacteria persist and escalate, the quest for viable and safe alternatives to traditional antibiotics becomes ever more pressing, with honey emerging as a potentially promising solution.

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