Journal of Pharmacy

Stingless Bee Honey Stick Deodorant: Formulation, Antioxidant and Antimicrobial Activities

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Abstract

Introduction: Deodorant often contain ingredients like aluminium salts, triclosan, fragrances, propylene glycol, and parabens that are usually associated with skin irritation and other health conditions. This study addresses the problem by formulating and characterising a natural deodorant that was free from these ingredients by using stingless bee honey (SBH) as an antibacterial and antioxidant ingredient. SBH, recognised for its efficacy in inhibiting the proliferation of odour-producing bacteria like *Staphylococcus sp*, was integrated into a stick deodorant formulation owing to its significant benefits. **Methods:** The evaluation of SBH began with testing its antioxidant activity, including total phenolic content (TPC), total flavonoid content (TFC) and DPPH assay. Thereafter, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Based on the MIC value, an SBH-stick deodorant was formulated and then characterised based on its pH, softening point, antimicrobial study by using well diffusion method and stability study (30 °C ± 2 °C /75% RH ± 5%) for two weeks. **Results:** The TPC and TFC in this study were 57.99 ± 0.38 mg GAE/100g and 0.132 ± 0.04 mg QE/mL, respectively. Meanwhile, the DPPH scavenging activity was 66.78 ± 0.45%. The result showed that 20% w/w and 50% w/w of SBH were needed as MIC and MBC, respectively. The formulated stick deodorant was reported to have suitable pH, softening point and exhibit its antibacterial activity towards *Staphyloccoccus aureus* after being formulated as deodorant. It was also stable during the two weeks of storage. **Conclusion:** The SBH stick deodorant was successfully formulated and demonstrated potential antibacterial activity against *Staphylococcus aureus*, a known body-odour causing bacteria. These findings highlight the potential application of SBH as a natural antibacterial agent in personal care products, offering a promising alternative to synthetic deodorants.

Article history:

Received: 15 Feb 2025
Accepted: 1 July 2025
Published: 31 July 2025

Keywords:

Stingless bee honey
Deodorant
Antioxidant
Antimicrobial
Kelulut

doi: 10.31436/jop.v5i2.386

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Introduction

Body odour, which mainly caused by activity of apocrine sweat glands, is influenced by various factors, including sex, diet, age, and genetics (Baker, 2019). Sweat is originally odourless, but body odour arises when bacteria on the skin come in contact with sweat, breaking down specific proteins in sweat into acids, leading to the generation of volatile organic compounds (VOCs), such as volatile fatty acids and thioalcohols. The primary bacteria involved in body odour formation are *Staphylococcus sp. Staphylococcus* bacteria were involved in body odour by metabolising compounds present in sweat, especially proteins and amino acids, into thioalcohols such as 3-methyl-2-hexenoic acid, which led to a pungent, unpleasant smell commonly associated with body odour. This explains the widespread use of deodorants and antiperspirant to manage body odour.

However, the use of antiperspirants and deodorants are linked to harmful effects due to certain ingredients. These include substances like aluminium salts (aluminium chloralhydrate or aluminium chloride) and zinc salts, which form gel plugs in sweat pores. Thus, it obstructs sweat from reaching the skin's surface and hinders the body's ability to eliminate toxins. Fragrances, propylene glycol, and parabens in these products are known allergens that may cause allergies and skin irritation (Sidek et al., 2021). Additionally, triclosan, an antibacterial compound utilised in personal care items, also raises concerns due to its oestrogen-disrupting properties (Farasani & Darbre, 2020). These concerns have led to a growing interest in natural alternatives.

SBH has been reported to exhibit significant antioxidant and antibacterial capabilities, covering for both gram-positive and gram-negative bacteria due to its phenolic and flavonoid compounds (Tuksitha et al., 2018), including *Staphylococcus sp.* in many studies (Ávila et al., 2019; Rosli et al., 2020; Tuksitha et al., 2018). Due to these properties, SBH is considered an excellent option to be used as an antibacterial ingredient in deodorants to eliminate body odour-causing bacteria, such as *Staphylococcus aureus*. Thus, this research aims to formulate and characterise a natural deodorant that is free from aluminium salts, triclosan, fragrances, propylene glycol, and parabens by using SBH as an antibacterial and antioxidant ingredient, which has not yet been explored in deodorant formulations.

Materials and methods

Materials

SBH, Heterotrigona itama (Kuin Honey, Kuantan, Malaysia), Folin–Ciocalteu reagent (Merck KGaA, Darmstadt, Germany), gallic acid (R&M Chemical Company, Selangor, Malaysia), quercetin hydrate 95% (Arcos Organics, Selangor, Malaysia), potassium acetate (HmbG Chemical, Selangor, Malaysia) , aluminium chloride hydroxy hydrate (Bendosen, Selangor, Malaysia) 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) reagent (R&M Chemical Company, Selangor, Malaysia), ascorbic acid (Sigma-Aldrich, St. Louis, United States), ethanol, Cetostearyl alcohol (R&M Chemical Company, Selangor, Malaysia), Olivem 1000 (Hallstar), isoamyl laurate (Future Food, Selangor, Malaysia), candelilla wax (Kahl, Trittau, Germany), zinc ricinolate (Take it Global, Penang, Malaysia), arrowroot powder (Take it Global, Penang, Malaysia), essential oil, vitamin E, Amoxicillin/clavulanic acid (Oxoid, Hampshire, United Kingdom), *Staphylococcus aureus* (ATCC 6538), Tryptic Soy Agar, Tryptic Soy Broth. All materials used in the formulation were cosmetic grade, except cetostearyl alcohol.

Method

Antioxidant Properties of SBH

Total phenolic content

The total phenolic content (TPC) was determined via spectrophotometry method. First, 1 mL of 0.2 g/mL honey was mixed with 1 mL of Folin–Ciocalteu reagent. After 3 minutes, 1 mL of sodium carbonate 10% and 7 mL of distilled water were added and mixed using a vortex mixer, then left to stand in the dark for approximately 90 minutes. The absorbance of the solution was measured at 725 nm, which corresponds to the

maximum absorbance of the blue complex formed by phenolic compounds with the Folin-Ciocalteu reagent. Gallic acid at concentrations of 0, 20, 40, 80, and 100 μg/mL were used as positive control. Phenolic content level was measured in triplicate and expressed as mg of gallic acid equivalents (GAEs) per 100 g honey (Khalil et al., 2012).

Total flavonoid content

The total flavonoid content (TFC) was determined using aluminium chloride colorimetric method. TFC test was conducted to determine the honey's ability to neutralise free radicals. First, a 50% v/v honey sample was prepared by diluting 2 mL of SBH with 2 mL of distilled water. Next, 1.5 mL of 95% ethanol was mixed with 500 μL SBH followed by 100 μL of 10% aluminium chloride hydroxy hydrate, 2.8 mL of deionised water and 100 μL of 1M potassium acetate. The solution was mixed and allowed to sit at room temperature for 30 minutes in the dark. Its absorbance was measured at a wavelength of 415 nm. Quercetin at concentrations 0, 20, 40, 60, 80, and 100 μg/mL were prepared as a standard (Tuksitha et al., 2018).

DPPH assay

The DPPH assay was conducted to measure the ability of honey to scavenge the free radicals. First, 50% v/v honey sample was prepared by diluting 2 mL of SBH with 2 mL of distilled water. Next, 300 μL of honey sample, 300 μL of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution and 2.4 mL of 99% ethanol were added and mixed before being left in the dark for 30 minutes. Next, the solution was centrifuged at 4500 rpm for 5 minutes, and the absorbance was measured at 517nm. Ascorbic acid as used as positive control. The percentage of free radical scavenging activity that targeted DPPH was calculated following Tuksitha et al., (2018) using Equation 1:

$$(\%) = [1 - (A_S/A_C)] \times 100 \quad (1)$$

Here, A_S is the absorbance of the honey sample while A_C is the absorbance of the control at 517 nm.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) procedure was conducted to determine the lowest concentration of honey sample that was able to inhibit the growth of the *Staphylococcus aureus* while minimum bactericidal concentrations (MBC) were used to identify the lowest concentration of honey that is required to kill the microorganism. An overview of these processes was illustrated in ***Fig 1.*** First, the optical density of the *Staph. aureus* was adjusted to 0.5 McFarland standard (1 to 2 × 10^8 cfu/ml) using a densitometer. A stock solution of SBH was prepared at a concentration of 70% w/w by dissolving 7 g of honey in 10 ml of tryptic soy broth (TSB). Next, the solution was filtered using 0.45μm filter to eliminate contaminating microorganisms before preparing serial dilutions of honey to achieve concentrations of 60%, 50%, 40%, 30%, 25%, 20%, 15%, 10% and 5% v/v.

In each 96-well plate, 190 μl of honey dilution was mixed with 10 μl of bacterial inoculum in triplicate for each dilution. A few control wells were prepared in triplicates which are 1) broth sterility control wells containing 200 μl of TSB alone, 2) viability control wells containing 190 μl of TSB and 10 μl of inoculum (without honey) and 3) dilution sterility controls containing 200 μl of the honey dilution in TSB (without inoculum). The plate was incubated overnight at 30 °C, and the absorbance of the wells was measured the following day at 590 nm. The percentage inhibition of bacterial growth was calculated using the formula in Equation 2:

$$Inhibition\ (\%) = 1 - \left(\frac{A_s - A_{dc}}{A_{vc} - A_{bc}}\right) \times 100 \quad (2)$$

Here, A_s is the absorbance of the test wells, A_{dc} is the absorbance of dilution sterility control, A_{vc} is the absorbance of viability control and A_{bc} is the absorbance of broth sterility control. The minimum value for the percent inhibition is 0% while the maximum value is 100%.

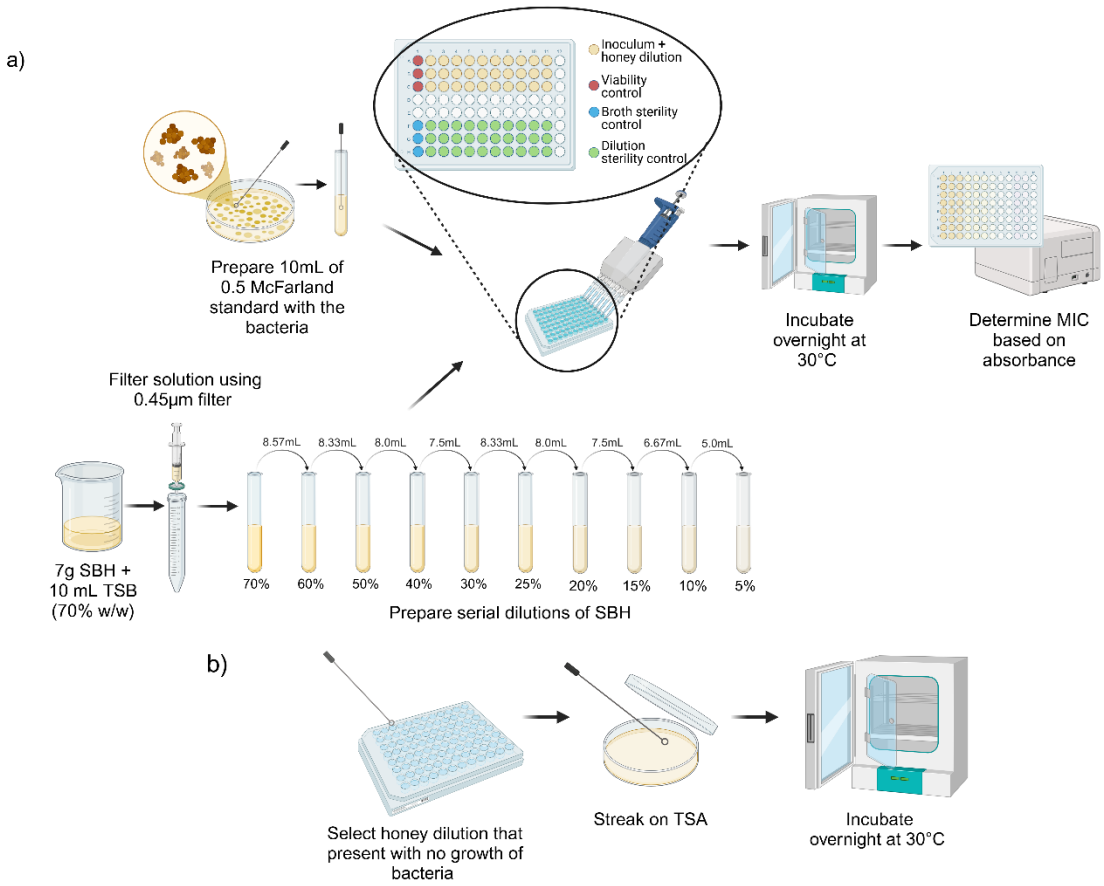

Fig. 1: Overview of (a) MIC and (b) MBC procedures

Minimum Bactericidal Concentration (MBC)

By using the streak plate method, honey dilution from each test well that showed no growth of bacteria in the MIC test was examined. A sterile wire loop was used to streak it onto the tryptic soy agar (TSA) plates. These plates were then incubated overnight at 30 °C. The minimal concentration of the diluted honey that showed no growth of the test inoculum (≥1%) was classified as the MBC (Zainol et al., 2013 as cited in Tuksitha et al., (2018).

Formulation of SBH Stick Deodorant

The ingredients were accurately weighed according to their respective percentages. Components in Phase A were combined and heated to 65 °C using a hot plate. The mixture was stirred with a magnetic stirrer until all components were fully melted and homogenised. Subsequently, the temperature was reduced to 45 °C before incorporating phase B ingredients. SBH added was

Table 1: Formulation of SBH stick deodorant

Phase	Ingredients	Function	w/w%
A	Cetostearyl alcohol	Emulsifier	15
	Olivem 1000	Emulsifier	10
	Isoamyl laurate	Emollient	17
	Beeswax	Thickening agent, binder	20
	Zinc ricinolate	Odour absorber	3
	Arrowroot powder	Thickening agent	14
B	Essential oils	Fragrance	0.8
	Vitamin E	Antioxidant	0.2
	SBH	Antibacterial	20*

* The honey added is based on the MIC value.

based on its MIC value (Komala et al., 2019), comprising 20% of the formulation, which is the lowest concentration of SBH required to effectively inhibit the growth of *Staphylococcus aureus*. This approach ensures optimal antimicrobial activity while minimising the use of SBH, thereby enhancing the cost-effectiveness. The mixture was then stirred for one minute. Finally, the prepared mixture was poured into containers and allowed to solidify at room temperature overnight. The formulation in Table 1 was labelled as F1. A blank of stick deodorant (F2), which had the same composition as F1, but did not contain SBH was also prepared to compare the antibacterial activity between the two deodorants.

Characterisation of Deodorant

Both F1 and F2 were characterised based on its pH, softening point, antimicrobial activity against *Staphylococcus aureus* and its stability at room temperature (30 °C ± 2 °C /75% RH ± 5%) for 14 days.

pH

The pH of the deodorant was measured to ensure it was suitable for application on underarm skin, which typically ranges from 4.0 to 6.8. First, 1% w/v sample was prepared by dissolving 1 g of stick deodorant into 100 mL of distilled water. The solution was heated to 40 °C and mixed using a vortex before being allowed to cool at room temperature. The pH was measured in triplicate using a calibrated pH meter (Insan & Vera, 2021).

Softening point

The softening point procedure was conducted following Debnath et al., (2011) method to determine the temperature at which the deodorant melted. The deodorant stick was cut in half lengthwise, mounted vertically in petri dishes, and placed in an oven. The temperature was then gradually increased until the sharp edges of the tip began to melt.

Antimicrobial Test

Antimicrobial test was conducted by following Debnath et al., (2011) with a few modifications. Antimicrobial test was conducted to make sure that the 20% of honey that was incorporated in the deodorant exhibits its antibacterial activities. First, 10% w/w of deodorant was prepared by dissolving 1 g of the deodorant into 9 mL distilled water. The optical density of the *Staphylococcus aureus* was adjusted to a 0.5 McFarland standard (1 to 2 × 10^8 cfu/ml) using a densitometer before swabbing it uniformly onto a nutrient agar plate with a sterile cotton swab. By using a borer, 0.5 cm wells were created in each plate and 0.15 mL of sample solution F1 and F2 was added into the well before incubating it at 37°C for 24 hours. The procedure was prepared in triplicate with amoxicillin/clavulanate as positive control. The inhibition zone was measured in triplicate and reported in centimetres (cm).

Stability test

Stability testing was conducted. A sample for each of the deodorant sticks, F1 and F2, was stored at room temperature at 30 °C ± 2 °C at 0, 7 and 14 days to measure the stability for both formulations. The room temperature conditions were controlled using an air-conditioned laboratory. The product was monitored for its organoleptic properties, including any signs of sweating, odour, changes in shape, colour, or separation of ingredients. If the stick lost its shape or exhibited the formation of oil droplets on the surface, it was deemed unstable.

Results and discussion

The antioxidant properties of SBH were assessed through the evaluation of its total phenolic content (TPC), flavonoid content, and DPPH scavenging activity and was demonstrated in **Table 2**.

Total phenolic content

The TPC of SBH was measured as 57.99 ± 0.3812 mg GAE/100g, indicating the presence of significant amounts of phenolic compounds, which were known for their antioxidant properties. The TPC value observed in this study was consistent with findings by Ya'akob et al. (2019), who reported that the phenolic content of SBH from eleven samples collected across different regions in Johor, Malaysia, ranged from 414.53 ± 3.166 mg GAE/kg to 778.23 ± 2.011 mg GAE/kg. Similarly, Ismail et al., (2021) reported TPC values for SBH derived from *Trigona sp* from Sabah and Kelantan ranged between 33.2 ± 1.2 to 60.2 ± 2.2 mg GAE/100 g. Thus, the TPC value in this study was expected to give positive effects in terms of antibacterial activity.

Table 2: Phenolic content and DPPH scavenging activity of SBH

Sample	Phenolic content		DPPH scavenging activity (%)
	TPC (mg GAE/100g)	TFC (mg QE/mL)	
SBH	57.99 ± 0.38	0.132 ± 0.04	66.78 ± 0.45

Note: The data are expressed as mean ± S.D. (n = 3)

Total flavonoid content

Meanwhile, the value of TFC for SBH in this study is 0.132 ± 0.04 mg QE/mL, which is considerably lower compared to the TFC values reported by Tuksitha et al. (2018), where three samples of SBH ranged from 12.41 ± 0.62 to 17.67 ± 0.75 mg QE/mL. However, the amount of TFC in the SBH is considered sufficient to provide antibacterial activity in this study.

DPPH assay

The DPPH scavenging activity of SBH in this study was measured at 66.78 ± 0.45%, which highlights the SBH's ability to scavenge free radicals. DPPH is a stable free radical. As the antioxidants in SBH donate their hydrogen atoms or electrons to DPPH, the free radical will reduce, resulting in the colour changes of DPPH from purple to yellow. This result was aligned with a study conducted by Mat Ramlan et al., (2021), which reported the percentage inhibition of eighteen samples of SBH from Malaysia and Australia approximately between 32.00% to 87.15%. However, this result was higher compared to Maringgal et al., (2019) and Tuksitha et al., (2018) which reported the percentage ranged between 2.77 ± 1.02% to 44.05 ± 11.04% and 17.0 ± 7.5% to 47.4 ± 3.2%, respectively. The variability of phenolic, flavonoid and antioxidant activities of SBH are due to factors such as bee species, geographical location, floral sources, and processing methods (Fatima et al., 2018; Shamsudin et al., 2019; Pimentel et al., 2021).

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The antimicrobial activity of SBH was then evaluated against *Staphylococcus aureus*, with the results presented in Fig. 2.

The MIC of SBH was found to be 20% w/w, indicating the lowest concentration required to completely inhibit visible bacterial growth of 1–2 × 10^8 cfu/mL. Meanwhile, the MBC was observed at 50% w/w, which represents the concentration necessary to eliminate bacterial growth at the same inoculum concentration.

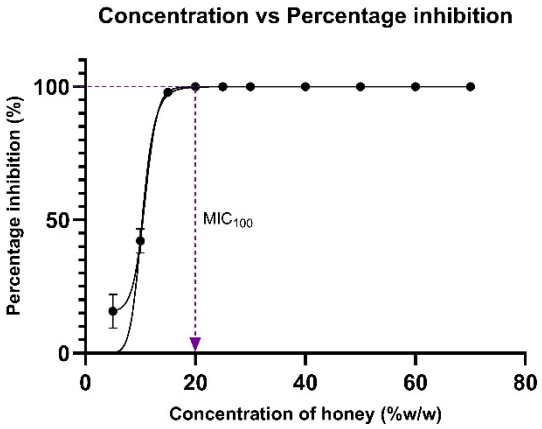

Fig. 2: Minimum Inhibitory Concentration (MIC) of SBH against *Staphylococcus aureus*.

In a study conducted by Mat Ramlan et al. (2021), it was reported that the MIC of three *Heterotrigona itama* honey samples from Malaysia and three *Tetragonula hockingsi* honey samples against *Staphylococcus aureus* ranged between 6–8% (w/w). These findings align with Sulaiman and Sarbon (2020), who reported a MIC of 6.25% (w/w), and Tuksitha et al. (2018), who reported that the MIC of SBH against *Staphylococcus aureus* ranged from 3% to 10% (w/w), while the MBC was slightly higher, ranged between 10% to 20% (w/w) across three different species of honey samples. As compared to the previous studies, the MIC and MBC levels for our honey sample are higher. This discrepancy can be attributed to the significantly higher bacterial density used in our study, which was 1–2 × 10^8 cfu/mL, compared to the bacterial density of 1 × 10^5 cfu/mL used in the previous studies. Thus, a higher concentration of honey is needed to effectively inhibit a higher concentration of *Staphylococcus aureus* growth, which results to higher MIC and MBC values.

According to studies, SBHs have stronger antibacterial properties than *Apis* honeybee honey (Rao et al., 2016; Zulkhairi Amin et al., 2018). SBH demonstrates considerable inhibitory effects against a broad spectrum of bacterial species, including gram-positive, gram-negative and multidrug-resistant strains (Ng et al., 2020; Tuksitha et al., 2018). The presence of its antibacterial activity may be influenced by a few factors. A review conducted by Nordin et al., (2018) revealed that SBH has high acidity due to its higher hydrogen ion in the honey composition, with pH ranges from 3.15 to 6.64. The

level of its acidity may be the contributor to bacterial fatality.

Other than that, studies correlate the antimicrobial properties with flavonoid content present in stingless bees, where flavonoids play a crucial role in antibacterial activity. These compounds disrupt membrane function and inhibit DNA synthesis, affecting the viability of pathogenic microorganisms. The antibacterial activity of flavonoids is closely linked to their chemical structure, particularly the hydroxy (OH) and methoxy (MeO) groups in phenolic rings. These structural components allow flavonoids to interact with bacterial proteins and membranes, leading to disruptions in the cell's structural integrity then, causing cell lysis and death (Komala et al., 2019). However, Biluca et al. (2016) reported no correlation between antimicrobial activity and antioxidant properties or phenolic compounds in honey samples. Similarly, Bueno-Costa et al. (2016) found no significant link between the TPC in honey from Rio Grande do Sul, Brazil, and its antibacterial activity against *Shigella dysenteriae, S. typhimurium, S. aureus*, and *Bacillus cereus* ($p > 0.05$).

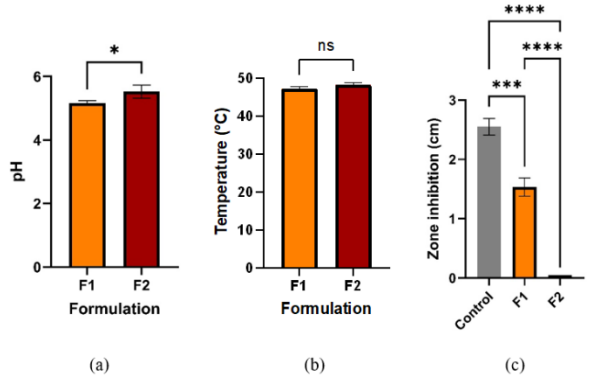

Fig. 3: (a) pH (b) Softening point (c) Zone of inhibition of stick deodorant against *Staphylococcus aureus.*

Formulation of SBH stick deodorant

The stick deodorant was subsequently formulated and characterized by evaluating key parameters, including its pH, softening point, and antimicrobial activity against *Staphylococcus aureus*, shown in **Fig. 3**. Additionally, a stability study was also conducted to assess its stability over time, as illustrated in **Table 2**.

During formulation process, SBH was added once the mixture had reached 45 °C. SBH are very sensitive to heat. In a study conducted by Mat Ramlan et al., (2021), SBH shows decreased antibacterial activities after being heated at 45 °C, 55 °C and 65 °C for an hour. However, another study conducted by Shahabuddin et al., (2022) reported that the antibacterial activity of SBH against *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella typhimurium* remained comparable to controlled honey when it was exposed to heat, 50°C for 10 minutes. Thus, incorporating the SBH in stick deodorant at a temperature of 45 °C for 1 minute in this study may help preserve its antibacterial activity while minimising the negative impact of prolonged exposure to heat. This temperature was also used to make sure that the mixture was still in the liquid phase to facilitate the pouring process.

Characterisation of Deodorant

pH

The pH of underarm skin typically ranges from 4.0 to 6.8, which is slightly broader than the general skin pH of about 4.5 to 6.5 (Komala et al., 2019). From the results, it shows that both pH of deodorant formulation that contains honey and without honey are 5.17 ± 0.08 and 5.53 ± 0.21 respectively. The addition of honey in the formulation causes a statistically significant difference ($p < 0.05$) between pH of the two formulations. However, both remain compatible with the natural underarm pH range. Based on Costa & Horswill (2022), an acidic skin pH offers stronger defence against harmful bacteria, including *Staphylococcus aureus,* which contribute to the formation of body odour. The bacteria grow optimally at pH 7.5 but exhibit reduced growth between pH 5.0 and 6.0. Thus, it can be concluded that the acidic pH of both deodorant formulations, particularly F1, not only aligns with the natural underarm pH but may also contribute to inhibiting the growth of *Staphylococcus aureus*.

Softening point

The softening point of the deodorant in **Fig 3(b)** with honey is 47.3 ± 0.6 °C, while the deodorant without honey has a slightly higher softening point of 48.3 ± 0.6 °C. The addition of honey in the formulation does not significantly ($p > 0.05$) affect the softening point of the stick deodorant. However, compared to commercial stick deodorant, as reported by Debnath et al., (2011) with a softening point of 66 °C, the values are considerably lower. This difference suggests potential challenges during handling and storage, especially in hot climates places.

Antimicrobial test

The antimicrobial activity of the stick deodorant was evaluated again against *Staphylococcus aureus* to assess its effectiveness. The results showed F1, which contains 20% w/w of honey, exhibited a statistically significant zone of inhibition (1.53 ± 0.15 cm, $p < 0.0001$), while the blank deodorant, F2 showed no inhibition activity. This indicates that SBH ingredients can still show antibacterial activity after being incorporated into the deodorant. Meanwhile, amoxicillin and clavulanate, which act as positive control show the biggest zone of inhibition measuring 2.55 ± 0.14 cm. In comparison to F1, this difference was statistically significant ($p < 0.001$). This result was aligned with a study by Rosli et al., (2020), where the zone inhibition for SBH for eight species was reported between 10 ± 0.00 cm to 28 ± 0.58 cm.

Stability study

Table 2. Stability study of stick deodorant

Day	Organoleptic properties (Colour, odour, sweating, melting)	
	F1	F2
Day 0	 **Fig. 4:** Organoleptic properties of formulations F1 The colour is yellowish, has a good rose smell, no visible signs of sweat on the surface, and in a solid form	 **Fig. 5**: Organoleptic properties of formulations F2 The colour is yellowish, has a good rose smell, no visible signs of sweat on the surface, and in a solid form
Day 7	No changes	No changes
Day 14	No changes	No changes

Based on the results shown in Table 2, the stick deodorant for both formulations remained stable during the two-week observation period at room temperature. The colour remained yellowish with rose aroma attributed to the beeswax and fragrance composition in the formulation. There were no visible signs of sweating or melting as they remained in solid form. The absence of sweating and phase separation indicates that the formulations were effectively emulsified and structurally stable.

Conclusion

The SBH stick deodorant was successfully formulated and demonstrated potential antibacterial activity against *Staphylococcus aureus*, a known body-odour-causing bacteria. For future studies, testing the deodorant on human participants is recommended to evaluate its practicality and effectiveness. Additionally, a roll-on deodorant could be considered instead of stick deodorant as it would avoid the heating process during production, which helps retain the natural properties of SBH. Lastly, the formulated deodorant can also be compared with commercially available deodorants to evaluate its suitability in the market further.

Authors contributions

Study design, S.A.N.Z. and M.M.D.M. Direction and Coordination, M.S.H. Investigation, S.A.N.Z. Resources M.S.H and S.M.R. Writing-Original Draft, S.A.N.Z. Writing-Review and Editing M.S.H., S.M.R., A.F.H.I and S.A.N.Z. Project Administration M.S.H. and S.A.N.Z.

Acknowledgements

The author would like to thank the Department of Pharmaceutical Technology Kulliyyah of Pharmacy, International Islamic University Malaysia and Quality Control Microbiology Laboratory, IKOP Pharma for providing facilities to complete the study.

Conflict of interest

The authors declare that there is no conflict of interest in writing this manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

ChatGPT was used to improve readability and language. The author then review and edit the content as needed. Turnitin was used to check plagiarism for this study.

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