

Evaluation of the Antimicrobial Efficacy of *Hibiscus sabdariffa* L. Antiperspirant Preparations Against *Staphylococcus epidermidis*

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Abstract

Commercial antiperspirant products commonly incorporate synthetic compounds, several of which have raised concerns due to their potential carcinogenicity. Moreover, the inclusion of natural antibacterial agents in these formulations remains limited. *Hibiscus sabdariffa* L. (Rosella), a plant rich in bioactive secondary metabolites, offers a promising natural alternative. This study aimed to evaluate the antibacterial efficacy of rosella-based antiperspirant formulations, particularly against *Staphylococcus epidermidis*, a key contributor to body odor. Three formulations (F1, F2, and F3) were developed and assessed based on several parameters: pH, organoleptic properties, homogeneity, spreadability, adhesiveness, and antibacterial activity against *Staphylococcus epidermidis*. The results demonstrated that the antiperspirant preparations complied with standard evaluation criteria. Furthermore, the antibacterial assay outcomes yielded statistically significant differences ($p < 0.05$) in the mean diameter of the inhibition zones, indicating that *H. sabdariffa* L. exhibited measurable antibacterial activity against *S. epidermidis*. These findings support the potential application of Rosella extract in developing natural and efficacious anti-perspirant products.

Keywords: Antiperspirant, *Hibiscus sabdariffa* L., Rosella, *Staphylococcus epidermidis*

INTRODUCTION

In the contemporary era, personal hygiene and body odor have emerged as critical aspects of maintaining individual cleanliness and significantly influencing self-image. Unpleasant body odors often result in a decline in self-confidence and may cause discomfort to those in close proximity to the individual (Inaku & Yusriani, 2024). Excessive perspiration can cause a range of issues, one of the most prominent being the emergence of an unpleasant body odor (Handayani et al., 2022). Sweat produced by the body plays a significant role in the development of body odor, particularly when the apocrine glands become susceptible to bacterial colonization, which facilitates decomposition and leads to the formation of malodorous compounds (Lailiyah et al., 2019). Body odor arises from the interaction between sweat and bacteria (Zahara, 2018).

The mechanism of antiperspirant action involves the precipitation of proteins present in sweat, along with the formation of keratin deposits within the epidermal layer, resulting in the occlusion of sweat duct walls through infiltrate development. Deodorants

prevent and neutralize body odor by inhibiting the bacterial activity responsible for sweat decomposition (Haerani et al., 2024).

Several bacterial species, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium acne* (diphtheroids), *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*, contribute to body odor development. Among these, *Staphylococcus* species can convert specific amino acids into short-chain volatile fatty acids, such as isovaleric acid, which emits a strong, unpleasant odor and plays a significant role in malodor formation, particularly in the axillary region (Siskawati et al., 2014).

Commercial deodorants are generally limited in their use of natural antibacterial agents (Haerani et al., 2024). Furthermore, a prevailing concern is the widespread use of synthetic compounds in deodorant products available in the market, some of which are suspected to act as potential carcinogens, particularly in relation to breast cancer risk. This concern arises from perspiration inhibition, which may interfere with the natural detoxification processes in the body (Rahmanda et al., 2023).

Rosella (*Hibiscus sabdariffa* L.), a species native to Asia and Africa, is a promising natural-based deodorant alternative. Ethanolic extracts of Rosella flowers contain many bioactive compounds, including flavonoids, saponins, tannins, phenols, polyphenols, triterpenoids, and glycosides. Flavonoids, such as gossypetin and hibiscetin, are particularly abundant in the calyces of flowers and contribute to plants' antimicrobial and antioxidant properties (Febriyanto et al., 2019; Suniarti et al., 2022). Rosella flower extract has been shown to exhibit pronounced antibacterial activity against *Staphylococcus aureus*, indicating its potential efficacy as a natural antimicrobial agent in topical formulations such as deodorants (Febriyanto et al., 2019). However, current studies primarily focus on the antibacterial activity of *H. sabdariffa* extract against *Staphylococcus aureus* and do not explore its effectiveness against *Staphylococcus epidermidis*, which also plays a significant role in body odor development (Hilda Sinaga, 2019; Lam et al., 2018; Lusida et al., 2017). This study focuses on formulating a natural deodorant incorporating *H. sabdariffa* L. extract and evaluates its antibacterial efficacy against *Staphylococcus epidermidis*, a microorganism associated with body odor that has received relatively limited attention in previous research. Therefore, this study aimed to develop a natural deodorant formulation incorporating ethanolic extract of *H. sabdariffa* L. flower and evaluate its antibacterial activity against *Staphylococcus epidermidis*.

Although numerous studies have examined the antibacterial activity of deodorant formulations, research focusing specifically on the incorporation of *Hibiscus sabdariffa* L. extract remains limited, particularly regarding its effectiveness against *Staphylococcus epidermidis*, a predominant bacterium involved in axillary odor. To address this gap, the present study formulated a natural deodorant using ethanolic extract of *H. sabdariffa* L. and evaluated its antibacterial potential against *Staphylococcus epidermidis*, aiming to support the development of safer, plant-based alternatives for personal care applications.

METHODOLOGY

Materials and Instrumentals

The instruments and apparatus employed in this study include: a set of laboratory glassware, analytical balance, UV-Vis spectrophotometer (Genesys), pH meter, rotary evaporator (IKA), incubator (Mettler), centrifuge, mortar and pestle, blender, hotplate, ose, petri dishes, autoclave, and inoculating loop.

The samples and materials used in this study included Rosella flowers (*H. sabdariffa* L. L.), specifically selected for their red coloration, freshness, and cleanliness, 96% ethanol (Merck), Carbopol 940, triethanolamine (TEA), butylated hydroxytoluene (BHT), distilled water (Aquadest), and the bacterial isolates *Staphylococcus epidermidis* ATCC-12228, Mueller Hinton Agar (MHA) (Oxoid), sterile sodium chloride (NaCl) solution, and chloramphenicol (30 µg/disk).

Methods

Sample Extraction

H. sabdariffa petals were initially subjected to sanitation and then rinsed under a sustained stream of water. The petals were air-dried and finely milled into a homogeneous powder (Oktaviani, 2021). A total of 100 g of *H. sabdariffa* L. flower powder was accurately weighed and subjected to maceration in 96% ethanol and maintained under sealed conditions for an approximate duration of five days. The ethanolic extracts were subsequently concentrated under reduced pressure using a rotary evaporator at 50°C and a rotational speed of 100 rpm, yielding a viscous ethanolic concentrate (Idrus et al., 2021; Suniarti et al., 2022).

Preparation of Deodorant Cream Formulated with *H. sabdariffa* L. Extract

The cream deodorant was prepared by adding half of the total volume of distilled water to a glass beaker. The gelling agent, Carbopol 940, was then incorporated into the aqueous phase and hydrated completely. Triethanolamine (TEA) was gradually introduced with continuous stirring until a homogeneous gel was obtained, and the pH was adjusted to approximately 6. In the second phase, the oil was prepared by melting Lexemul CS20, butylated hydroxytoluene (BHT), and zinc ricinoleate at a controlled temperature of 80°C. After complete melting, the mixture was stirred until homogeneity was achieved. The remaining distilled water was heated separately to 70°C to dissolve aluminum potassium sulfate. Subsequently, the aqueous phase was incrementally added to the oil phase under continuous agitation to ensure proper emulsification. Once the mixture became uniform, phenoxyethanol and the gel prepared from the first phase were incorporated and stirred until complete homogeneity was achieved. Finally, *H. sabdariffa* L. extract was added to formulations F1, F2, and F3, and the mixtures were stirred thoroughly until a consistent and uniform cream was formed (Putri et al., 2023).

Table 1. Antiperspirant Formulation

Bahan	Formula (%)			
	F0	F1	F2	F3
Rosella Extract	-	5	15	25
Zinc ricinoleate	2	2	2	2
Aluminium potassium sulfate	2	2	2	2
Carbopol 940	0.5	0.5	0.5	0.5
TEA	0.5	0.5	0.5	0.5
Lexemul CS20	5	5	5	5
BHT	0.1	0.1	0.1	0.1
Phenoxyetanol	1	1	1	1
Aquadest	88.9	83.9	73.9	36.1

Antibacterial Test of Deodorant Cream Formulated with *H. sabdariffa* L. Extract

Mueller Hinton Agar (MHA) medium (20–25 mL) was poured into sterile Petri dishes, and 0.2 mL of the bacterial suspension was added. Once the medium solidified, 10 μ L of the test preparation was carefully added. Antibacterial activity was tested on deodorant formulations F1, F2, and F3. A commercial deodorant was the positive control, whereas F0 was the negative control. The inoculated plates were incubated at 37°C for 24 h. After incubation, the inhibition zones, evident as clear halos around the sample sites, were measured using calipers to determine the antibacterial efficacy of each formulation (Putri et al., 2023).

Organoleptic and Physicochemical Evaluation of Deodorant Cream Formulated with *H. sabdariffa* L. Extract

a. Organoleptic Test

Samples from each cream deodorant formulation were evaluated based on organoleptic parameters, including texture, color, odor, and homogeneity (Mayangsari et al., 2023).

b. pH Test

A 0.5 g sample was diluted in 5 mL of distilled water for pH analysis. According to SNI 16-4951-1998, the acceptable pH range for cream deodorants is 4–6, aligning with skin compatibility standards (Mayangsari et al., 2023; Megantara et al., 2017).

c. Homogeneity Test

Homogeneity was evaluated by spreading 0.5 g of the cream on a watch glass. A formulation was deemed homogeneous if it appeared uniform and free from particles or granules (Lidia et al., 2022).

d. Spreadability Test

To assess spreadability, 0.5 g of cream was placed between two glass plates and subjected to incremental weights (20–125 g), with the diameter of the spread recorded after one minute for each load. The procedure

was repeated three times, with a target spreadability range of 5–7 cm (Lidia et al., 2022).

e. Adhesiveness Test

A sample was compressed between two plates with a 1 kg load for 5 minutes, followed by applying of a 100 g weight to initiate separation. The detachment time was recorded and averaged over three trials to evaluate adhesiveness (Megantara et al., 2017).

Data analysis

The data obtained from the antibacterial activity tests were analyzed using SPSS software version 24. The Kruskal-Wallis test was used to determine whether there were statistically significant differences in the inhibition zone diameters among the various deodorant formulations with different extract concentrations. A *p*-value less than 0.05 was considered indicative of a statistically significant difference.

RESULTS AND DISCUSSION

Phytochemical screening was conducted to identify classes of secondary metabolites with potential biological activity in the Rosella extract (Alemu et al., 2024; Farhamzah & Khofifah, 2018; Rosmawaty & Tehubijuluw, 2013). The results of the phytochemical screening of the Rosella flower extract are presented in Table 2.

Table 2. Phytochemical Result of *Hibiscus sabdariffa* L. Extract

Phytochemical Constituents	Result
Alkaloid	+
Flavonoid	+
Triterpenoid	+
Steroid	+
Saponin	+
Tannin	+

According to the organoleptic evaluation of the Rosella flower antiperspirant preparations, no discernible differences were observed in color, odor, and homogeneity across formulations F1, F2, and F3. However, there was notable variation in texture, wherein formulation F3 exhibited comparatively greater fluid consistency than formulations F1 and F2. The results of the organoleptic test are presented in Table 3.

Table 3. Organoleptic test result

Formulation	Organoleptic			Homogeneity
	Color	Odor	Texture	
F0	White	Odorless	Soft, easily spreadable, semi-solid	Homogen
F1	Magenta-Purple	Typical of Rosella	Soft, easily spreadable, semi-solid	Homogen
F2	Magenta-Purple	Typical of Rosella	Soft, easily spreadable, semi-solid	Homogen
F3	Magenta-Purple	Typical of Rosella	Easily spreadable, Semi-Liquid	Homogen

Table 4. The Result of pH, Spreadability and Adhesiveness Test

Formulation	pH	Spreadability (cm)	Adhesiveness
F0	4.56 ± 0.012	4.02 ± 0.602	1.21 m ± 0.297
F1	3.22 ± 0.177	4.40 ± 0.103	5.20 m ± 0.200
F2	3.12 ± 0.321	4.07 ± 0.350	5.25 m ± 1.127
F3	2.85 ± 0.362	4.06 ± 0.077	0.34 s ± 0.191

Table 4 shows that the pH assessment of each formulation yielded distinct values, with formulation F3 presenting the lowest mean pH of 2.85 ± 0.362 , which deviates slightly from the normal physiological skin pH, typically ranging from 4 to 6 (Mayangsari et al., 2023). An observable trend indicated that increasing concentrations of Rosella extract corresponded with a progressive decline in pH. This acidification effect is presumably due to the inherent acidic constituents of the Rosella extract, such as hibiscus acid (Sedillo-Torres et al., 2022), which, when incorporated in greater amounts, imparts heightened acidity to the final formulation. To evaluate pH, topical formulations must possess a pH similar to the skin, to minimize the potential irritation and to ensure dermal compatibility (Timur & Latifah, 2019). The results of this study indicate that although the *Hibiscus sabdariffa* L. extract exhibited antibacterial activity against *Staphylococcus epidermidis*, the overall formulation of the antiperspirant requires further optimization to ensure its compatibility with the skin environment.

The spreadability evaluation aimed to elucidate the capacity of the cream base to disperse uniformly across the dermal surface, thereby indicating the ease of

topical application. An optimal spreadability profile facilitates broader contact between the active substance and the skin, enhancing the rate and efficiency of transdermal absorption (Pratasik et al., 2019). The minimum requirement for spreadability of a topical preparation is conventionally set at 5-7 cm² (Timur & Latifah, 2019). The highest spreadability value was observed in formulation F1, which exhibited a dispersion diameter of 4.40 cm. Nevertheless, this result remains below the established minimum threshold for topical preparations, indicating that the formulation does not fulfill the requisite spreadability criterion.

To evaluate the tenacity of the cream upon dermal application, an adhesiveness assay is imperative to determine the temporal threshold necessary for the formulation to affix itself effectively to the skin, as shown in Table 5. Topical pharmaceutical preparations are conventionally required to exhibit a minimum adhesion time of no less than four seconds. Superior adhesiveness is beneficial because it facilitates sustained contact with the epidermal surface and minimizes the likelihood of inadvertent detachment (Pratasik et al., 2019). The results obtained from formulations F1 and F2 demonstrated markedly superior adhesiveness, with retention times of 5.20 minutes and 5.25 minutes, respectively. In contrast, formulation F3 exhibited an adhesion duration of less than 4 s, failing to meet the established criteria. This insufficiency may be ascribed to the relatively more fluid consistency of F3, which likely compromises its ability to maintain prolonged contact with the skin surface.

Table 5. Evaluation of the Antimicrobial Efficacy of Rosella's Antiperspirant Formulations Against *Staphylococcus epidermidis*

Formulation	Inhibition Zone (mm)
F0	10.47 ± 0.275
F1	13.22 ± 1.234
F2	12.85 ± 0.433
F3	15.58 ± 0.252
Control (+)	13.23 ± 0.535

The ability of rosella flowers to inhibit bacterial growth cannot be separated by the content of its active compound (Hayati et al., 2023). The mechanism of action of antibacterial active compounds involves several sequential steps, including disruption of the bacterial protoplasm, penetration and damage to the bacterial cell wall, and precipitation of intracellular proteins. These processes collectively impair essential

bacterial functions, inhibiting growth and ultimately leading to cell death (Mulyono et al., 2023).

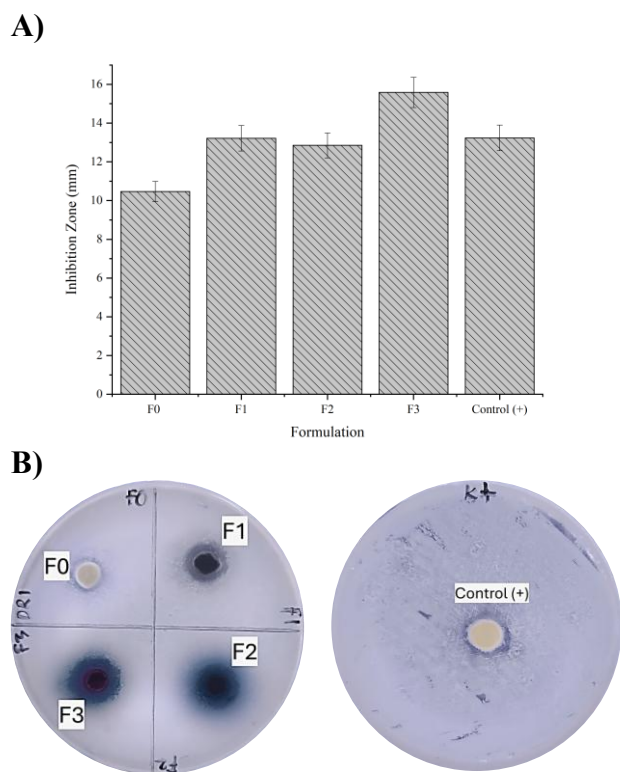


Figure 1. Antibacterial Activity of Rosella's Antiperspirant. (A) Inhibition Zone of Antiperspirant Formulation; (B) Antibacterial capacity

The ethanolic extract of *H. sabdariffa* L. exhibited significant antibacterial activity against *S. epidermidis* with inhibition zones of F1, F2, and F3 of 13.22 ± 1.234 , 12.85 ± 0.433 , and 15.58 ± 0.252 , respectively (Figure 1). An X commercial antiperspirant used as a positive control in the study exhibited inhibition zone values of 13.23 ± 0.535 against *S. epidermidis*. This indicates the potency of the commercial antiperspirant in inhibiting bacterial growth at a relatively low value compared to the plant extract antiperspirant (Figure 1) (Alemu et al., 2024).

The antibacterial activity of *H. sabdariffa* L. may be attributed to various bioactive compounds, such as hibiscus acid (Sedillo-Torres et al., 2022). These compounds possess antimicrobial properties and have been found to disrupt membrane integrity and increase the permeability of bacterial cells. In addition to hibiscus acid, other flavonoids in *H. sabdariffa* L. may contribute to its antibacterial activity. Flavonoids possess antioxidant and anticarcinogenic activities and exhibit antibacterial properties (González et al., 2016; Mere et al., 2021).

The inhibitory mechanisms of flavonoid compounds on nucleic acid synthesis involve either intercalation into DNA strands or forming hydrogen bonds with nucleic acids, ultimately leading to nucleic acid accumulation and suppression of DNA and RNA biosynthesis. The B-ring of the flavonoid structure is particularly critical in this interaction. Regarding membrane disruption, flavonoids form complexes with extracellular proteins and soluble constituents, causing structural damage to the cell membrane and resulting in the leakage of intracellular contents. Additionally, flavonoids interfere with cellular energy metabolism by altering membrane permeability, disrupting the proton electrochemical gradient (proton motive force) across the membrane. This gradient is vital for ATP synthesis and membrane transport; thus, disturbance of this gradient by flavonoids leads to a decrease in ATP generation and impairment of essential transport mechanisms (Cushnie & Lamb, 2005; Farhamzah & Khofifah, 2018; Mulyono et al., 2023). Other secondary metabolites present in rosella may also contribute to its antibacterial activity. For instance, tannins can exert antimicrobial effects by forming complexes with nucleophilic proteins through non-specific interactions, including hydrogen and covalent bonding. Terpenoids can penetrate microbial cell membranes and interact with vital intracellular targets. Additionally, the antibacterial action of saponins is attributed to their membrane-disrupting properties and their ability to lower surface tension in the extracellular environment (Khan et al., 2025).

Statistical analysis performed using the Independent Samples Kruskal-Wallis Test in SPSS yielded a p-value of 0.023 ($p < 0.05$), indicating a statistically significant difference in the diameter of inhibition zones among the deodorant formulation groups. This finding suggests that variations in the extract concentration within each formulation significantly influence antibacterial activity against *Staphylococcus epidermidis*.

CONCLUSION

The antiperspirant formulation incorporating *Hibiscus sabdariffa* L. extract demonstrated notable antibacterial efficacy against *Staphylococcus epidermidis*, with the F3 formulation exhibiting the most substantial inhibitory zone, measuring 15.58 ± 0.252 mm, thereby indicating pronounced antibacterial activity.

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