

## Exploring the Antibacterial Potential of Traditional Plants for Body Odor Control

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### ABSTRACT

Engaging in physical activities such as exercise or sports often results in sweating, which is considered a healthy practice for promoting well-being. However, excessive sweating during daily life, especially when accompanied by unpleasant odors, can indicate poor hygiene and lead to discomfort and social problems. Deodorants and antiperspirants are commonly used to eliminate body odor, but their potential side effects have encouraged the search for natural alternatives. This study investigated the antibacterial properties of ten medicinal plant extracts traditionally used to mitigate body odor. Using ethanol extracts, the antibacterial activity was assessed through agar well diffusion and microbroth dilution methods against common axillary bacteria, such as *Staphylococcus epidermidis*, *Corynebacterium tuberculo-stearicum*, and *Corynebacterium jeikeium*. The plant extracts showed diverse antibacterial effects, with inhibition zones ranging from  $0.0 \pm 0.0$  to  $16.33 \pm 0.57$  mm. The MIC and MBC values were recorded between 1.563 and 0.098 mg/mL. Extracts of *Piper betle*, *Syzygium aromaticum*, and *Curcuma xanthorrhiza* showed significant effects in inhibiting the growth of bacteria responsible for body odor, indicating their potential as natural components in deodorant and antiperspirant formulations.

**Keywords:** Antimicrobial, Body odor, Deodorant, Medicinal plants, Sweating

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### Introduction

Body odor is not merely a superficial issue; it deeply impacts various aspects of life, including emotional, social, work-related, psychological, and physical well-being. Studies indicate that people affected by body odor tend to experience higher levels of anxiety compared to the general population and those with other chronic health problems. While medical treatments like botulinum toxin injections and surgical procedures are available, many individuals still favor the use of deodorants and antiperspirants. This preference has contributed to the growing market, which reached an estimated value of 74.55 billion U.S. dollars in 2019 [1].

Despite their widespread use, deodorants and antiperspirants are associated with potential health risks due to their chemical contents. For example, aluminum chloride, commonly found in antiperspirants, has been linked to an increased risk of breast cancer [2]. Triclosan, an antimicrobial compound often included in personal care products, is recognized for its ability to interfere with estrogen, raising concerns about its possible connection to breast cancer [3-5]. Additionally, animal studies have shown that triclosan can contribute to hypothyroidism by disturbing thyroid hormone levels through the activation of the Pregnane-X-receptor (PXR) and inhibiting diiodothyronine (T2) sulfotransferases [6]. Other frequently used substances, including fragrances, propylene glycol, and parabens, have been flagged as potential allergens [7]. As a result, there is an increasing interest in natural alternatives.

In Southeast Asia, there is a long-standing tradition of utilizing medicinal plants to address body odor and excessive perspiration. Despite this tradition, there is a lack of research on the antibacterial properties and effectiveness of these plants. Some studies have examined natural deodorants made from plants such as *Salvia*

*officinalis* (sage) [8] and *Eugenia caryophyllus* (clove) [9]. This research seeks to investigate the antibacterial activity of ten medicinal plants that have been traditionally used to control body odor, specifically assessing their impact on the skin microbiota responsible for odor formation.

#### *Preparation to extract plants*

A total of ten plant species, including *Piper betle* L., *Pluchea indica* (L.) Less, *Ocimum basilicum* L., *Curcuma xanthorrhiza* Roxb., *Etlingera elatior* (Jack) R. M. Sm., *Citrus hystrix* D.C., *Citrus aurantifolia* (Christm.) Swingle, *Zingiber officinale* Roscoe, *Cucumis sativus* L., and *Syzygium aromaticum* (L.) Merr. & L.M. Perry were collected from Malaysia. These plants were chosen for their traditional use in addressing body odor and excessive sweating. Each plant sample weighed approximately 5 kg and was freshly harvested to ensure the preservation of their natural compounds. The plants were cleaned thoroughly, rinsed to remove any dirt or contaminants, and then cut into smaller pieces to facilitate the extraction process.

Once cleaned, the plant material was subjected to shade-drying to preserve its bioactive compounds, which could degrade if exposed to direct sunlight or excessive heat. After drying, the plant material was finely ground into a powder to increase the surface area, allowing for more efficient extraction of the active ingredients.

The extraction process involved maceration, a technique where the powdered plant material is soaked in a solvent—in this case, ethanol. The ethanol-to-plant material ratio used was 1:20 (w/v), meaning 1 gram of plant material was soaked in 20 milliliters of ethanol. This soaking process lasted for three days at room temperature, allowing the active compounds to dissolve into the ethanol. Afterward, the liquid extract was filtered to remove any solid residues from the plant material. To ensure a more thorough extraction, the process was repeated three more times with fresh ethanol on the remaining plant material, allowing the maximum amount of active compounds to be extracted.

The liquid extracts from each extraction were collected and combined into a single solution, which was then concentrated by evaporating the ethanol under reduced pressure. This step removed the solvent, leaving behind a more concentrated extract. The resulting plant extracts were measured to determine their final yield and then stored at 4 °C to maintain their stability for future analysis or use in further experiments. This method ensures the preservation of the bioactive compounds, allowing for the potential development of natural deodorant and antiperspirant formulations.

#### *Antibacterial assay*

##### *Bacterial strains*

The antibacterial activity of the plant extracts was assessed against three gram-positive bacterial strains: *Staphylococcus epidermidis* (ATCC 14990), *Corynebacterium tuberculostrictum* (ATCC 35693), and *Corynebacterium jeikeium* (ATCC 43734). These bacterial strains are commonly associated with body odor production.

##### *Inoculum preparation*

Each bacterial strain was cultured on sterilized agar plates and incubated at 37 °C for 24 hours. After this incubation, a single bacterial colony was transferred into a test tube containing 10 mL of sterile saline solution. The bacterial suspension was thoroughly mixed using a vortex to ensure even distribution. The concentration of the bacteria was then standardized to match a 0.5 McFarland turbidity standard.

##### *Agar well diffusion*

The plant extracts were prepared at a final concentration of 50 mg/mL by dissolving them in 5% DMSO. To test the antibacterial activity, bacterial suspensions were evenly spread onto Mueller Hinton (M.H.) agar plates using a sterile cotton swab. The spreading process was repeated three times, rotating the plate each time to achieve uniform distribution of the inoculum. Three 6 mm wells were created on the agar surface using sterile Durham tubes. Each well was filled with 50 µL of the following: plant extract (50 mg/mL), the positive control (gentamicin at 1 mg/mL), or the negative control (5% DMSO). The agar plates were incubated aerobically at 37 °C for 24 hours. Each experiment was conducted in triplicate to ensure reproducibility.

#### *Specification of minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC)*

The plant extracts were dissolved in 5% DMSO and diluted to the highest concentration. A series of two-fold serial dilutions were prepared directly into 96-well microplates containing Mueller Hinton (M.H.) broth, achieving a concentration range from 50.0 mg/mL to 0.0977 mg/mL. Each well was inoculated with 50  $\mu$ L of bacterial suspension ( $5 \times 10^8$  CFU) of each bacterial strain. Positive controls, ranging from 1000  $\mu$ g/mL to 62.5  $\mu$ g/mL, negative controls (5% DMSO with 100  $\mu$ L of inoculum), and environmental controls containing only the media were included and tested in triplicate. The plates were incubated aerobically at 37 °C. Bacterial growth was monitored by measuring turbidity and observing pellet formation using a UV-Vis spectrophotometer at 625 nm. The Minimum Bactericidal Concentration (MBC) was identified as the lowest concentration of the plant extract where no bacterial growth was detected after incubation.

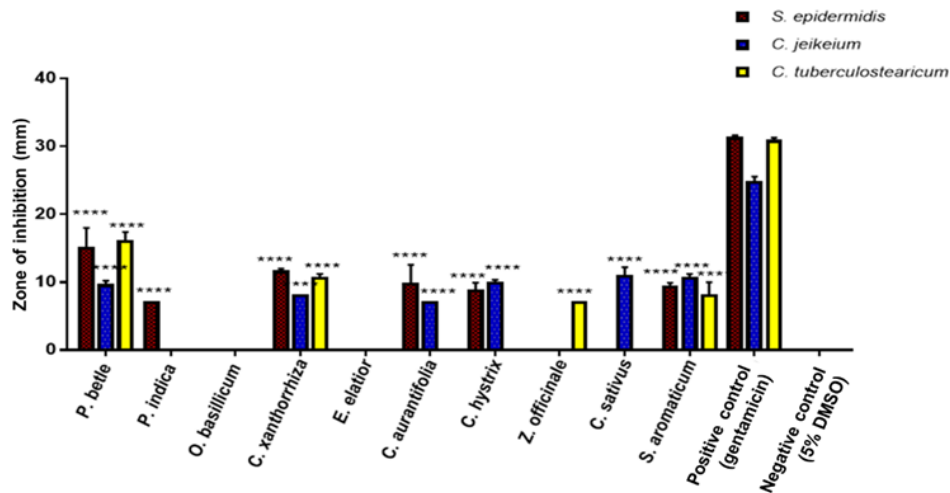
## Results and Discussion

Deodorants and antiperspirants make up a significant segment of the global health and beauty industry, which is expected to reach USD 92,707 million by 2024. Concerns have emerged about their potential link to breast cancer, with a particular focus on the estrogenic effects of parabens as a potential cause. While several studies support this theory, others challenge it. Additionally, increasing worries about the risks of ingredients such as aluminum chloride and triclosan have led to a rise in research for natural alternatives. This study explores the antimicrobial properties of ten medicinal plants traditionally used for body odor control, assessing their effectiveness against three specific skin microbiota species responsible for body odor, aiming to identify safe and effective components for deodorants and antiperspirants (**Table 1**).

**Table 1.** Ethnobotanical data and extract yield percentage of selected medicinal plants

Plant Species	Family	Plant part used	Dry weight (g)	Ethanollic extract (g)	Extract yield (%)
<i>Syzygium aromaticum</i> (L.) Merr & L.M. Perry	Myrtaceae	Flower Bud	100.0	28.4	28.4
<i>Curcuma xanthorrhiza</i> Roxb.	Zingiberaceae	Rhizome	100.0	22.7	22.7
<i>Piper betle</i> L.	Piperaceae	Leaf	70.0	12.4	17.7
<i>Citrus aurantifolia</i> (Christm.) Swingle	Rutaceae	Fruit	50.0	8.4	16.8
<i>Etlingera elatior</i> (Jack) R. M. Sm.	Zingiberaceae	Flower	20.0	3.1	15.5
<i>Citrus hystrix</i> DC	Rutaceae	Fruit	50.0	6.25	12.5
<i>Pluchea indica</i> (L.) Less	Asteraceae	Leaf	80.0	5.5	6.88
<i>Ocimum basilicum</i> L.	Lamiaceae	Whole Plant	50.0	4.5	9.00
<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Rhizome	100.0	8.9	8.90
<i>Cucumis sativus</i> L.	Cucurbitaceae	Fruit	10.0	0.8	8.00

The antibacterial activity of the plant extracts was assessed using well diffusion assays to measure their zones of inhibition (**Table 2**), and the minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) were calculated (**Table 3**). Among the ten plants tested, *Piper betle*, *Curcuma xanthorrhiza*, and *Syzygium aromaticum* showed strong inhibition against all three bacterial strains, while *Ocimum basilicum*, *Etlingera elatior*, and *Cucumis sativus* had no effect (**Figure 1**). Significant differences were found in the inhibition zones between the extracts and the negative control ( $P < 0.05$ ). The antibacterial activity of *Piper betle* is mainly attributed to its phenolic compounds, which disrupt bacterial cell walls and membranes, particularly in Gram-positive bacteria, leading to their breakdown. Previous studies have shown its effectiveness against foot odor-causing bacteria like *S. epidermidis* and *Bacillus subtilis*, making *Piper betle* a promising natural solution for foot odor management.



**Figure 1.** Average zone of inhibition (mm) of 10 plant extracts against selected body odor-causing bacteria (n = 3, Mean  $\pm$  SD); \*\*\*\* P < 0.0001 compared to positive control.

**Table 2.** Zone of inhibition (mm) of plant extracts against selected bacteria causing body odor (Plant extract concentration: 50 mg/mL)

Plant extract	<i>S. epidermidis</i>	<i>C. tuberculostearicum</i>	<i>C. jeikeium</i>
<i>Piper betle</i> L.	15.0 $\pm$ 3.0	16 $\pm$ 1.41	9.5 $\pm$ 0.71
<i>Pluchea indica</i> (L.) Less	7.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>Ocimum basilicum</i> L.	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>Curcuma xanthorrhiza</i> Roxb.	11.5 $\pm$ 0.50	10.5 $\pm$ 0.71	8.0 $\pm$ 0.0
<i>Etlingera elatior</i> (Jack) R. M. Sm.	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>Citrus aurantifolia</i> (Christm.) Swingle	9.67 $\pm$ 2.89	0.0 $\pm$ 0.0	7.0 $\pm$ 0.0
<i>Citrus hystrix</i> DC	8.75 $\pm$ 1.17	0.0 $\pm$ 0.0	9.80 $\pm$ 0.57
<i>Zingiber officinale</i> Roscoe	0.0 $\pm$ 0.0	7.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>Cucumis sativus</i> L.	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>Syzygium aromaticum</i> (L.) Merr & L.M. Perry	16.33 $\pm$ 0.57	16.0 $\pm$ 2.0	10.5 $\pm$ 0.71
Positive control (gentamicin)	30.33 $\pm$ 0.49	26.67 $\pm$ 0.83	32.41 $\pm$ 0.51
Negative control (5% DMSO)	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

The data illustrates the antibacterial activity of various plant extracts against bacteria responsible for body odor. It displays the inhibition zones for each bacterial strain (*S. epidermidis*, *C. tuberculostearicum*, and *C. jeikeium*) following treatment with the plant extracts. Gentamicin, the positive control, demonstrated strong antibacterial effects, while the negative control (5% DMSO) showed no antibacterial activity.

*Curcuma xanthorrhiza*, specifically its rhizome, contains bioactive compounds such as curcumin and xanthorrhizol, which possess broad-spectrum antibacterial properties effective against both Gram-positive and Gram-negative bacteria [10]. Curcumin works by interfering with bacterial cell division, altering the permeability of the bacterial cell membrane, and allowing for the uncontrolled movement of substances across the membrane [11]. This disrupts the internal cellular environment by causing the leakage of essential substances like ions, enzymes, amino acids, and nutrients. The loss of these critical components hampers bacterial metabolism and reduces the availability of adenosine triphosphate (ATP), which is essential for bacterial growth and reproduction, ultimately leading to bacterial cell death [12].

The antibacterial properties of *Syzygium aromaticum* are attributed to eugenol, a phenolic compound [13, 14]. Eugenol disrupts the bacterial membrane, inhibiting bacterial growth [15]. Previous studies have shown that

*Syzygium aromaticum* extract effectively inhibits the growth of Gram-positive bacteria, including *S. aureus* and *S. epidermidis* [16].

Among the three plant extracts tested, *Curcuma xanthorrhiza* demonstrated the strongest antibacterial effects across all the bacterial strains: *S. epidermidis* (0.0977 mg/mL), *C. tuberculostearicum* (0.391 mg/mL), and *C. jeikeium* (0.195 mg/mL) (**Table 3**). The Minimum Inhibitory Concentration (MIC) was used to determine the concentration at which no bacterial growth was observed, and the Minimum Bactericidal Concentration (MBC) was established by identifying the lowest MIC that resulted in the absence of bacterial growth (**Table 3**). MBC analysis indicated the bactericidal potential of *Piper betle* extract against *S. epidermidis* and *C. jeikeium*; *Curcuma xanthorrhiza* extract against *S. epidermidis* and *C. tuberculostearicum*; and *Syzygium aromaticum* extract against *C. tuberculostearicum*.

**Table 3.** MIC and MBC of key plant extracts against bacteria linked to body odor

Plant extracts	<i>S. epidermidis</i>	<i>C. tuberculostearicum</i>	<i>C. jeikeium</i>
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)
<i>Piper betle</i> L.	0.391	0.391	0.781
<i>Curcuma xanthorrhiza</i> Roxb.	0.098	0.098	0.391
<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry	0.098	0.195	0.781
Positive Control (gentamicin)	0.004	0.004	0.031

While *Piper betle* and *Syzygium aromaticum* showed promising antibacterial activity against skin-associated microbes, concerns have been raised regarding eugenol, one of their key active compounds. At high concentrations, eugenol has been associated with several adverse effects. Research has indicated that dental products containing eugenol can lead to skin irritation, dermatitis, tissue damage, and delayed wound healing [17-19].

## Conclusion

In conclusion, this study highlights the strong antibacterial potential of *Piper betle*, *Syzygium aromaticum*, and *Curcuma xanthorrhiza* extracts against body odor-causing bacteria. These plants offer promising natural alternatives to synthetic deodorants and antiperspirants, addressing health concerns and the growing demand for sustainable personal care solutions. The results suggest the potential for plant-based formulations to provide safe, eco-friendly, and non-toxic options for managing body odor and excessive sweating.

However, while *Piper betle* and *Syzygium aromaticum* showed effective antibacterial properties, the side effects of their active compound, eugenol, require further investigation. Future research should focus on optimizing these extracts for personal care products, assessing their long-term safety, and exploring alternatives to reduce risks associated with eugenol.

This study underscores the importance of utilizing natural resources in personal hygiene solutions, benefiting both personal and environmental health.

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