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ANTIMICROBIAL POTENTIAL OF TEN MEDICINAL PLANT EXTRACTS AGAINST AXILLARY MICROBIOTA CAUSING BODY ODOR

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ABSTRACT

Sweating after a workout or playing sports is an acceptable form of promoting well-being. However, excessive sweating in normal life activities, especially when associated with a foul odor, reflects poor hygiene, leading to embarrassment and social barriers. Individuals suffering from these problems require continuous maintenance using deodorants and antiperspirants as the most common treatments for body odor. However, the association of these products with side effects increases the need to search for antiperspirant and deodorant from natural resources. Ten medicinal plant extracts traditionally used to mask body odor were screened against skin microbiota for antibacterial activity. Agar well diffusion and microbroth dilution method were utilized to evaluate the antibacterial activity of the ethanol extract of the plants against bacteria strains commonly found on the axillary region; *S. epidermidis*, *C. tuberculostearicum*, and *C. jeikeium*. The plant extracts have shown varying antibacterial activity, with the zone of inhibition for susceptibility tests ranging from 0.0 ± 0.0 to 16.33 ± 0.57 mm. The MBC and MIC of the plants against the investigated bacterial strains were 1.563 to 0.098 mg/mL. Ethanol extracts of *Piper betle*, *Syzygium aromaticum*, and *Curcuma xanthorrhiza* inhibited the growth of three strains of skin microbiota that causing body odor and thus, indicated as a promising medicinal plant in the development of natural deodorants and antiperspirant.

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Introduction

Body malodor was not just cosmetically inconvenient but had profound effects on emotional, social, occupational, psychological, and physical disorder in a considerable number of people. The affected individuals have been shown to have a higher prevalence of anxiety symptoms than what is usually reported among the general population and in patients with other chronic diseases. Despite several modern intervention methods like botulinum toxin injection and local surgery, many still use deodorant and antiperspirant. The never-ending need for these lifestyle products has led to the expansion of this industry, and it was evaluated to be worth about 74.55 billion U.S. dollars in 2019 [1].

However, the usage of antiperspirants and deodorants is associated with certain side effects of the ingredients in the products. One study suggested that aluminum chloride present in most antiperspirants today can cause breast cancer [2]. Another compound, triclosan (2,4,40-trichloro-20-hydroxydiphenyl ether), an antimicrobial agent, is extensively used in personal care products. It has estrogen-disrupting features that increase worries for engagement in breast cancer [3-5]. Furthermore, triclosan is also associated with hypothyroidism in rats at which triclosan alters the thyroid hormone homeostasis through activation of the human Pregnane-X-receptor (PXR) and inhibition of diiodothyronine (T2) sulfotransferases [6]. Besides, fragrance, propylene glycol and parabens in deodorants and antiperspirants are found to be allergenic [7]. This has influenced the society to seek for natural solutions to this problem.

There are many medicinal plants traditionally known to counter the problem of body odor and excessive sweating within the South-East Asia region. Despite their potential, very limited studies on the activity and efficacy of these natural ingredients were available. Among the works were the formulation analysis of natural deodorant sticks from *Salvia officinalis* (sage) [8]

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and *Eugena caryophyllus* (clove) [9].

The present research aimed to investigate the in vitro antibacterial activity of ten medicinal plants traditionally used to reduce body odor against skin microbiota responsible for body odor.

Materials and Methods

Preparation of Plant Extracts

5 kg of fresh selected plant samples (10 species)- *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 obtained from a cultivation farm in Pahang, Malaysia. Plants were washed, rinsed, cut into pieces, and allowed to dry under shade. The dried plants were ground into powdery form. The maceration method produced Crude ethanol extracts with the ratio of 1:20 (w/v) at room temperature for 3 days. The extracts were filtered, and the process was repeated thrice on the residue. The filtrates were combined, and the ethanol was removed under pressure. The extract's yield was weighted and stored at 4°C until used.

Antibacterial Assay

Bacterial Strains

The antibacterial activity of each plant extracts was investigated by three bacteria strains that were known to cause body odor. Following the standard bacterial strains were used in this study belonging to Gram-positive species; *Staphylococcus epidermidis* (ATCC 14990), *Corynebacterium tuberculostearicum* (ATCC 35693), and *Corynebacterium jeikeium* (ATCC 43734).

Inoculum Preparation

The tested bacteria strains were cultured separately on sterilized agar at 37°C for 24 hours. A single colony of the bacteria was moved into a sterilized test tube with 10 ml sterile normal saline solution. The suspension of bacteria was mixed well uniformly using a vortex. The suspension of each bacterium was regulated with 0.5 McFarland turbidity standards.

Agar Well Diffusion

To make a final concentration of 50 mg/mL, the extracts were dissolved in 5% DMSO. Bacteria suspensions were swabbed onto Mueller Hinton (M.H.) agar and then evenly seeded and streaked using a sterile cotton swab on the agar plate surface. The process was redone by streaking three times, rotating the plate each time to assure a balanced distribution of inoculums. A sterilized Durham tube is used to make 3 wells of 6 mm on the agar. 50 µL of plant extract (50mg/mL), positive control (gentamicin, 1mg/mL), and negative control (5% DMSO) were poured into each well and were then incubated at 37°C aerobically for 24 hours. The experiments were carried out for three times.

Specification of the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC)

The extracts were dissolved in 5% DMSO and diluted at the highest concentration. Two-fold dilutions were prepared directly into 96-well microplates containing M.H. broth to obtain a concentration range of 50.0 to 0.0977 mg/mL. 50 µL of 5x108 cfu of each bacterium were loaded in each well. The positive controls (concentration range 1000 µg/mL - 62.5 µg/mL), negative controls (5% DMSO with 100µL inoculum), and environmental control of media were also prepared and analyzed in a triplet. The plates were aerobically incubated at 37°C. Bacteria growth was indicated by turbidity evaluated by a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) at wavelength 625nm and the formation of a pellet. The lowest condensation of the extract revealed no bacterial growth after incubation was seen and observed as the MBC.

Statistical Analysis

The findings were demonstrated by means ± standard deviation. The data were analyzed using one-way variance (ANOVA) analysis in GraphPad Prism 7 Software (C.A., USA) accompanied by Bonferroni's multiple comparison post-test. Differences were regarded significant if the value was $p < 0.05$.

Results and Discussion

Deodorants and antiperspirants make up one of the largest segments in the health and beauty industry, with the global market are anticipated to reach USD 92,707 million by 2024 [10]. Both products have received much attention as the possible cause of increasing breast cancer, with most hypotheses indicating parabens' estrogenic properties as the main contributing factor [11]. Numerous studies supporting and discrediting this claim have been published [12]. Besides, alarming concerns on the association of other main active ingredients used in deodorants and antiperspirants, like aluminum chloride and triclosan, are among other risks that have led to the increasing research and development of deodorants of natural origin in recent years.

Regarding many antimicrobial studies conducted on plant extracts reviewed, this study focuses on screening ten medicinal plants traditionally used to reduce body odor against three specific species of skin microbiota responsible for causing body odor, intending to produce a clinically safe and effective ingredient for deodorant and antiperspirant.

The botanical data of the selected medicinal plants and their extract percentage yield is recorded in **Table 1**. The highest yield of the plant extracts was obtained from *Syzygium aromaticum* (28.4%), followed by *Curcuma xanthorrhiza* (22.7%), while *Pluchea indica L*. (6.88%) gave the lowest extract yield.

Table 1. Ethnobotanical Data of Selected Medicinal Plants and their Yield Percentage

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

The activity of these extracts against selected bacteria causing body odor was assessed based on their zone of inhibition (mm) in well diffusion assays **(Table 2)** and further quantified using MIC and MBC **(Table 3)**. Out of the ten medicinal plants screened, the extracts of *Piper betle*, *Curcuma xanthorrhiza*, and *Syzygium aromaticum* showed good inhibition against all three tested bacteria strains. In contrast, no inhibition was observed for *Ocimum basillicum*, *Etlingera elatior*, and *Cucumis sativus* **(Figure 1)**. The extracts showed a significant difference in the zone of inhibition against all tested bacteria in contrast to the negative control ($p < 0.05$). The phenolic compounds in *Piper betle* extract gave its bacteriostatic and bactericidal effects [13, 14]. Phenol causes damage in three-dimensional proteins and covalent structural differences in Gram-positive bacteria and leads to destruction in the bacteria's cell walls [15]. Flavonoid also contributes to the antibacterial activity of the Piper betle extract by interfering with the potassium concentration on Gram-positive bacteria, leading to disruption and dysfunction of the cytoplasmic membrane. Previous studies have demonstrated the antibacterial activity of *Piper betle* against *S. epidermidis* and *Bacillus subtilis*, which are microorganisms known to be the main cause of foot odor [16, 17]. *Piper betle* extract can also be used as an active ingredient to eliminate foot odor [18].

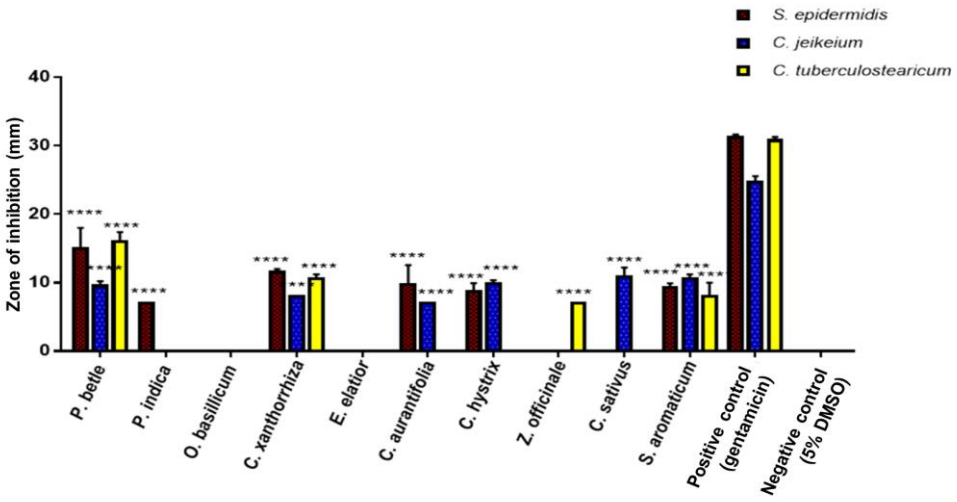

Figure 1. Average Zone of Inhibition (mm) of 10 Plant Extracts against Selected Bacteria Causing Body Odor. (n=3, Average ± SD), **** $p<0.0001$ compared to Positive Control

Table 2. Zone of Inhibition (mm) of Plant Extracts against Selected Bacteria causing Body Odor

Plant extract (50 mg/mL)	**Inhibition zone (mm)**		
	S. epidermidis	***C. tuberculo-stearicum***	***C. jeikeium***
Piper betle L.	15.0 ± 3.0	16 ± 1.41	9.5 ± 0.71
Pluchea indica (L.) Less	7.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Ocimum basillicum L.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Curcuma xanthorrhiza Roxb.	11.5 ± 0.50	10.5 ± 0.71	8.0 ± 0.0
Etlingera elatior (Jack) R. M. Sm.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Citrus aurantifolia (Christm.) Swingle	9.67 ± 2.89	0.0 ± 0.0	7.0 ± 0.0
Citrus hystrix DC	8.75 ± 1.17	0.0 ± 0.0	9.80 ± 0.57

Zingiber officinale Roscoe	0.0 ± 0.0	7.0 ± 0.0	0.0 ± 0.0
Cucumis sativus L.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Syzygium aromaticum (L.) Merr & L.M. Perry	16.33 ± 0.57	16.0 ± 2.0	10.5 ± 0.71
Positive control (gentamicin)	30.33 ± 0.49	26.67 ± 0.83	32.41 ± 0.51
Negative control (5% DMSO)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

For *Curcuma xanthorrhiza*, the secondary metabolite compound in the rhizome, mainly curcumin and xanthorrhizol, have broad-spectrum antibacterial activity against Gram-negative and Gram-positive bacteria [19]. Curcumin works by inhibiting cell proliferation, altering the permeability of the bacterial cells, and causing the uncontrollable movement of substances in and out of the cell [20]. Substances in the cell such ad organic ions, enzymes, amino acids, and nutrients leave the bacterial cell. When enzymes leave the bacterial cells along with the substances such as water and nutrients, there will be an inhibition of metabolism and result in a decrease in adenosine triphosphate (ATP) required for the growth and proliferation of the bacterial cells. Eventually, bacterial cell growth inhibition occurs and leads to death in the bacterial cell [21].

The antibacterial activity of *Syzygium aromaticum* is due to the existence of eugenol, a phenolic compound [22, 23]. Eugenol cause disruption to the bacterial membrane and in turn result in inhibition growth of bacteria [24]. The previous studies showed that Syzygium aromaticum extract showed good antibacterial activity against Gram-positive bacteria, S. aureus, and S. epidermidis [25].

Among the three extracts, *Curcuma xanthorrhiza* shown the highest antibacterial activity against all tested bacteria strains S. epidermidis (0.0977 mg/mL), *C. tuberculostearicum* (0.391 mg/mL), and *C. jeikeium* (0.195 mg/mL) **(Table 3)**. The MBC was confirmed based on no growth observation on the tested bacteria strains from the lowest MIC **(Table 3)**. The extract of MBC analysis showed potential bactericidal activity 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 the Most Influential Plant Extract against Bacteria Causing Body Odor

Plant extracts	***S. epidermidis***		***C. tuberculo-stearicum***		***C. jeikeium***	
	MIC (mg/mL)	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
Piper betle L.	0.391	0.391	0.781	1.563	1.563	1.563
Curcuma xanthorrhiza Roxb.	0.098	0.098	0.391	0.391	0.195	0.391
Syzygium aromaticum (L.) Merr & L.M. Perry	0.098	0.195	0.781	0.781	0.781	1.563
Positive control (gentamicin)	0.004	0.004	0.031	0.031	0.016	0.016

Although *Piper betle* and *Syzygium aromaticum* showed potential to inhibit the growth of skin microbial, concerns were raised on one of the major active compounds for both plants, eugenol. Several side effects were associated with a high concentration of eugenol [26]. The use of dental products containing eugenol was observed to irritate the skin, dermatitis allergy, necrosis tissue, and delaying wound healing [27, 28].

Conclusion

Piper betle, *Syzygium aromaticum*, and *Curcuma xanthorrhiza* inhibited the growth of three strains of skin microbiota that causing body odor and thus, indicated as a promising medicinal plant in the development of natural deodorant and antiperspirant.

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Conflict of interest: None

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Ethics statement: Design of the experiments was approved by UKM Animal Ethics Committee with the approval number FF/2018/MAZLINA/28-NOV./970-NOV.02018-SEPT.-2019.

References

1. Sabanoglu T. Size of the global antiperspirant and deodorant market 2012-2025. Stat Mark Res. [Internet] 2020. [cited on 25 May 2021]. Available from: www.statistica.com.
2. Linhart C, Talasz H, Morandi EM, Exley C, Lindner HH, Taucher S, et al. Use of underarm cosmetic products in relation to risk of breast cancer: a case-control study. EBioMedicine. 2017;21:79-85.

3. Farasani A, Darbre PD. Long-term exposure to triclosan increases migration and invasion of human breast epithelial cells in vitro. J Appl Toxicol. 2021;41(7):1115-26. doi:10.1002/jat.4097
4. Abdulsahib WK, Fadhil OQ, Abood SJ. Antimicrobial susceptibility pattern isolated from different clinical samples in Baghdad hospitals. J Adv Pharm Edu Res. 2020;10(1):51-9.
5. Aloqbi AA. Gum Arabic as a natural product with antimicrobial and anticancer activities. Arch Pharm Pract. 2020;11(2):107-12.
6. Zhang P, Yang M, Zeng L, Liu C. P38/TRHr-Dependent Regulation of TPO in Thyroid Cells Contributes to the Hypothyroidism of Triclosan-Treated Rats. Cell Physiol Biochem. 2018;45(4):1303-15.
7. Bouslimani A, da Silva R, Kosciolek T, Janssen S, Callewaert C, Amir A, et al. The impact of skin care products on skin chemistry and microbiome dynamics. BMC Biol. 2019;17(1):47. doi:10.1186/s12915-019-0660-6
8. Mohammad AS, Mustafa G, Ali F, Niloufar S, Dariush S, Syed AF. Deodorant effects of a sage extract stick: Antibacterial activity and sensory evaluation of axillary deodorancy. J Res Med Sci. 2013;18(10):833-9.
9. Debnath S, Babu MN, Kusuma G. Formulation and Evaluation of Herbal Anti-microbial deodorant Stick. Res J Topic Cosmet Sci. 2011;2(1):21-4.
10. Antiperspirant and Deodorant Market by Function (Aerosol Spray, Cream, Roll-On, and Gel), by Ingredient (Alcohol, Antimicrobials, Conditioner & Moisturizer, Fragrances, Paraben & Propellants, and Others): Global Industry Perspective, Comprehensive Analysis, and Forecast, 2017—2024; Zion Market research [Internet], 2019. [Cited on: 25 May 2021] Available from: https://www.zionmarketresearch.com/report/antiperspirant-and-deodourant-market.
11. Linhart C, Talasz H, Morandi EM, Exley C, Lindner HH, Taucher S, et al. Use of underarm cosmetic products in relation to risk of breast cancer: A case-control study. EBioMedicine. 2017;21:79-85.
12. Antiperspirants/Deodorants and Breast Cancer. National Cancer Institute Factsheet [Internet], 2017. [Cited on 15 May 2021]. Available from: https://www.oncologynurseadvisor.com/home/cancer-types/breast-cancer/antiperspirants-deodourants-and-breast-cancer-fact-sheet/.
13. Syahidah A, Saad CR, Mohd DH, Rukayadi Y, Norazian MH, Kamarudin MS. Phytochemical analysis, identification and quantification of antibacterial Active Compounds in Betel Leaves, Piper betle Methanolic Extract. Pak J Biol Sci. 2017;20(2):70-81.
14. Sravanthi P, Archana G. Phytochemicals and their antimicrobial activity: An update on their mode of action. Int J Clin Exp Med Res. 2021;5(1):41-69.
15. Cetin-Karaca H. Evaluation of Natural Antimicrobial Phenolic Compounds against Foodborne Pathogens. University of Kentucky Master's Thesis [Internet] 2011; 652. [Cited on 25 May 2021]. Available from: https://uknowledge.uky.edu/gradschool_theses/652
16. Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial activity of flavonoids and their structure-activity relationship: An update review. Phytother Res. 2019;33(1):13-40.
17. Farhamzah, Herli A, Mursal ILP. Formulation and antibacterial activity test of foot spray with beluntas leaf ethanol extract (Pluchea Indica L.). IOP Conf Ser: Mater Sci Eng. 2021;1071(1):012013.
18. Dorkbuakaew N, Jitpukdeebodintra S. Evaluation of Betel Leaf Extract Deodourizing Foot Spray. Thaksin Univ J. 2019;22(1):43-51.
19. Rahmat E, Lee J, Kang Y. Javanese Turmeric (Curcuma xanthorrhiza Roxb.): Ethnobotany, Phytochemistry, Biotechnology, and Pharmacological Activities. Evidence-Based Complementary and Alternative Medicine. 2021;2021. doi:10.1155/2021/9960813
20. Mekni MA, Achour W, Hassen AB. Overview of genetic background beyond Polysaccharide Intercellular Adhesion Production in Staphylococcus Epidermidis. Jundishapur J Microbiol. 2017;10(1):1.
21. Ngadino S, Koerniasari E, Sa S. Evaluation of Antimycobacterial Activity of Curcuma xanthorrhiza Ethanolic Extract against Mycobacterium Tuberculosis H37rv in Vitro. Vet World. 2018;11(3):368.
22. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. Syzygium aromaticum L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. Biomolecules. 2020;10(2):202. doi:10.3390/biom10020202
23. Da Silva FF, Monte FJ, de Lemos TL, Do Nascimento PG, de Medeiros Costa AK, De Paiva LM. Eugenol derivatives: synthesis, characterization, and evaluation of antibacterial and antioxidant activities. Chem Cent J. 2018;12(1):1-9.
24. Ulanowska M, Olas B. Biological properties and prospects for the application of eugenol-A review. Int J Mol Sci. 2021;22(7):3671. doi:10.3390/ijms22073671
25. Sakha H, Hora R, Shrestha S, Acharya S, Dhakal D, Thapaliya S, et al. Antimicrobial activity of ethanolic extract of medicinal plants against human pathogenic bacteria. Tribhuvan Univ J Microbiol. 2018;5:1-6.
26. Okmen G, Mammadhkanli M, Vurkun M. The Antibacterial Activities of Syzygium aromaticum (L.) Merr. & Perry against Oral Bacteria and Its Antioxidant and Antimutagenic Activities. Int J Pharm Sci Res. 2018;9(11):4634-41.
27. Mohammadi NS, Özgüneş H, Başaran N. Pharmacological and toxicological properties of eugenol. Turk J Pharm Sci. 2017;14(2):201-6.
28. Sarrami N, Pemberton M, Thornhill M, Theaker E. Adverse reactions associated with the use of eugenol in dentistry. Br Dent J. 2002;193(5):257-9.