

## Article

# Antiperspirant and Antibacterial Activities of *Curcuma xanthorrhiza* Extract as a Potential Alternative Treatment for Hyperhidrosis

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**Abstract:** Hyperhidrosis is a condition characterized by excessive sweating that affects at least 5% of the world's population. It is normally associated with unpleasant body odour which requires continuous maintenance throughout life. Antiperspirants and deodorants are the first-line treatments for hyperhidrosis. However, association of this class of product with the risk of other diseases has increased the search for an effective antiperspirant from natural sources. This study aimed to investigate the antibacterial and antiperspirant activities of *Curcuma xanthorrhiza* Roxb. Using an in vitro microbial assay against specific bacteria responsible for body odour and rat foot pad analysis. Quality control of the fractionated extract was conducted using reversed-phase HPLC based on two active markers: xanthorrhiza and curcumin. Toxicity tests were conducted based on the OECD guidelines (50–2000 mg/kg). Inhibition zone, MIC, and MBC values of the hexane fraction of *C. xanthorrhiza* showed equivalent antibacterial activity to positive control ( $p > 0.05$ ). No swelling, redness, or rashes were observed at the injection area throughout the study. Treatment with 40, 80, and 160 mg/kg of the extract significantly decreased the sweat excretion up to 79.34% ( $p < 0.05$ ) after 15 min compared to negative control. Histopathology analysis showed the reduced size of the secretory units in plantar sweat glands post treatment compared to negative control. In conclusion, the hexane fraction of *C. xanthorrhiza* is able to reduce sweat excretion in a rat model and inhibit the growth of bacteria causing body odour.

**Keywords:** *Curcuma xanthorrhiza*; antimicrobial; antiperspirant; sweating; herbal medicines; body odour; deodorant



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## 1. Introduction

A skin disorder characterized by excessive sweating for more than what is required for thermoregulation is clinically known as hyperhidrosis. A study has shown that in primary focal hyperhidrosis, especially for individuals with axillary hyperhidrosis, sweating can have economic implications that affect workplace productivity [1]. It is not merely a cosmetic inconvenience but a serious, often disabling, medical condition that has profound effects on occupational, emotional, psychological, social, and physical impairment in a substantial proportion of individuals [2]. According to the International Hyperhidrosis Society, at least 5% of the world's population is affected by the disease which requires continuous maintenance throughout life [3]. However, the numbers demonstrated may be conservative due to underreported cases by patients and those underdiagnosed by healthcare professionals [1]. Another condition that can be associated with sweating is body odour. Sweat itself does not smell, but body odour may occur when bacteria on the skin break down acids contained in the sweat produced by apocrine glands.

Deodorants and antiperspirants are two of the most used cosmetic products, with millions of consumers applying these products to their axilla every day. Deodorants are used to mask odour, whereas antiperspirants are used to reduce the amount of sweat

produced. Both products make up one of the largest segments in the health and beauty aid industry with the global antiperspirant and deodorant market estimated to be worth about USD 74.55 billion in 2019 [4]. While deodorants are considered as cosmetic products because they do not change the function of the skin, antiperspirants are classified as drugs and are therefore subject to rules and regulations set forth by the Food and Drug Administration (FDA). The active ingredient in antiperspirants is usually aluminium-based, which reduces sweat by causing obstruction of the eccrine glands [5].

In spite of its popularity, the main ingredients used in conventional preparations garnered a lot of attention for having the risk of breast cancer and skin problems, including the main active ingredient, aluminium chloride [6–8], the antimicrobial agent that is extensively used in personal care products [9], triclosan [10], propylene glycol, and parabens [11]. Thus, a new effective and safe compound from natural resources is a desirable alternative.

In previous work, we screened the ethanol extracts of several Malaysian medicinal herbs known to neutralize body odour for their antimicrobial activity against three types of skin microbiota that cause this problem [12]. Despite the traditional claimed usage, there was very little clinical and safety research to back up these claims. Among the ten extracts evaluated, *Curcuma xanthorrhiza* Roxb was identified to have good activity against the skin microbials with a good safety profile. This finding, on top of the traditional usage and claim, has propelled this research into focusing on the potential of *C. xanthorrhiza* as a natural antiperspirant agent.

There were tremendous studies conducted on *C. xanthorrhiza*, especially on its antibacterial property. However, based on our review, antimicrobial analysis of *C. xanthorrhiza* extract on *Corynebacterium jeikeium*, *Corynebacterium tuberculostrictum*, and *Staphylococcus haemolyticus*, the skin microbiota causing body odour as used in this study, was never explored. Moreover, the antiperspirant effect of this plant has never been studied before. In this work, the antibacterial activity of different polarities of *C. xanthorrhiza* extracts were tested against four types of skin microbiota causing body odour followed by antiperspirant assays using rat foot pad methods. Quality control of the extract was conducted using RP-HPLC.

## 2. Materials and Methods

### 2.1. Plant Materials and Extraction Process

The fresh rhizome of *C. xanthorrhiza* was purchased from Pahang, Malaysia. The specimen (voucher no: UKMB40446) was identified by a botanist of Universiti Kebangsaan Malaysia (UKM) and deposited at the Herbarium of UKM, Bangi, Malaysia. An amount of 100 g dried rhizome of *C. xanthorrhiza* was macerated with ethanol with the ratio 1:5 (*w/v*) at room temperature for 3 days and filtered [13]. This process was repeated thrice. Filtrates were collected and evaporated using a rotary evaporator. The remaining water content was removed using a freeze-drying technique.

The extracts were sequentially fractionated using hexane, ethyl acetate and acetone as eluting solvents until a clear solvent was observed.

### 2.2. Quality Control by HPLC

HPLC analysis was conducted using Breeze with a dual  $\lambda$  absorbance detector (Waters 2487, Milford, MA, USA) isocratic HPLC pump (Waters 1515, Milford, MA, USA) equipped with an autosampler (Waters 717 plus, Milford, MA, USA) and C18 column (Xterra MS C18 5 $\mu$ m, 4.6 mm  $\times$  150 mm, Waters, USA). For the methods of identification and quantification of curcumin and xanthorrhizol, we referred to the Malaysian Herbal Monograph [14] and Aguilar et al. [15], respectively, with modification.

An amount of 0.5 mg/mL hexane fraction of *C. xanthorrhiza* extract was diluted to volume in ethanol. An amount of 1 mg/mL of stock solution for the standard compounds was prepared in methanol. For curcumin, the mobile phase used consisted of acetonitrile-orthophosphoric acid (0.1%) in water (60:40 *v/v*). The flow rate was adjusted to 0.5 mL/min, injection volume was 10  $\mu$ L and the detected wavelength was set at 425 nm. For xanthorrhizol, the mobile phase used was acetonitrile-water (85:15 *v/v*). The flow rate

used was 1.0 mL/min, injection volume was 10 µL and the detection wavelength was set at 230 nm [15].

### Analytical Validation for HPLC Quantification

The method was validated by determining its linearity, the limit of detection (LOD), and limit of quantification (LOQ) as conducted by Husain et al. [16]. The linearity was assessed by the calibration curve of the standards at the concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. Intraday precision was determined by injecting six replicates of one of the standards (100 µg/mL). Interday precision was determined by injecting a triplicate of three concentrations of the standards, which were 12.5, 25, and 50 µg/mL. The precision was determined by examining the RSD value for the retention time and peak area. LOD and LOQ were calculated based on the slope of the calibration curve using the following equation:  $LOD = 3.3 \times \text{standard deviation slope}$  and  $LOQ = 10 \times \text{standard deviation slope}$ .

### 2.3. Antibacterial Study

#### 2.3.1. Samples and Standards Preparation

An amount of 50 mg/mL of extracts was prepared by dissolving 100 mg of extract in 20 mL 5% DMSO. An amount of 1 mg/mL of Gentamicin was used as positive standard and 5% DMSO as negative standard.

#### 2.3.2. Tested Microorganism

Four bacteria strains associated with body odour formation were used in this study: *Staphylococcus epidermidis* (*S. epidermidis*; ATCC 14990), *Staphylococcus haemolyticus* (*S. haemolyticus*; ATCC 29970), *Corynebacterium jeikeium* (*C. jeikeium*; ATCC 43734), and *Corynebacterium tuberculoearicum* (*C. tuberculoearicum*; ATCC 35692).

#### 2.3.3. Inoculums Preparation

All the bacteria strains were inoculated and spread on prepared agar plates using a sterile inoculating wire loop and incubated for 24 h at 37 °C. Then, the bacteria turbidity of each species was prepared and standardized by following the guidelines of the Clinical and Laboratory Standard Institute (CLSI). The test suspension was standardized to match the 0.5 McFarland turbidity standard which corresponds to approximately  $1 \times 10^8$  CFU/mL.

#### 2.3.4. Antibacterial Assay

A well diffusion method was used for the antibacterial susceptibility test. A sterile cotton swab was dipped into the microbe suspension and swabbed on the agar plate surface three times by spinning the agar plate clockwise. The plate was left for a few minutes to let the inoculums be absorbed into the agar. The edge of the Durham tube was sterilized and used to make wells on the agar plate. An amount of 50 µL of extract solution, positive control, and negative control were pipetted into a well that had been punched. The plates were incubated at 37 °C for 24 h. The inhibition zone was observed and measured using the vernier calliper.

Determination of MIC was performed in 96-well microdilution plates. To each well of the microdilution plate, 100µL of medium was added. Then, in the first row of the plate, 100µL of 50 mg/mL of extract was added. Subsequent concentration of the extract was obtained through two-fold serial dilutions on the plate, starting from 50 mg/mL to 0.0122 mg/mL by transferring 100 µL of the contents of a well to the subsequent well. Finally, 100 µL of the contents of the wells in Row H was removed and discarded to level the total volume of each well to be same. To assess sterility, broth alone was added to the wells, as environmental control. Following this, 10 µL of bacterial suspension ( $1.5 \times 10^3$  CFU/mL) was added to each well, except those in the environmental control well. The plates were then incubated at 37 °C for 24 h.

After MIC identification, 10 µL of solution from each well without bacterial growth was spread over their respective agar and incubated at 37 °C for 24 h. Concentrations

that killed > 99.9% of the bacterial population and showed no bacterial colonies on agar after 24 h of the incubation period were recorded as MBC values. The experiment was conducted in triplicate.

## 2.4. In Vivo Toxicity Analysis

### 2.4.1. Sample Preparation

The hexane fraction of *C. xanthorrhiza* was placed in a vial and weighed. Appropriate amounts of 2% Tween 80 were added to each vial to achieve dose concentrations of 50, 100, 1000, and 2000 mg/kg for in vivo toxicity testing by weight for each rat tested. For this, 2% of aluminium chloride and 2% Tween 80 in WFI were used as positive and negative controls, respectively.

### 2.4.2. Experimental Animals

A total of 24 male Sprague Dawley rats (170–250 g) were obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, UKM. The rats were maintained and acclimatized under controlled temperature ( $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ), 12 h light/12 h dark conditions, for one week before the start of experiments. They were provided with water and food ad libitum. The rats were divided into 4 groups ( $n = 6$ ), receiving doses of 50, 100, 1000, and 2000 mg/kg of extracts in each group.

To assess the potential toxicity of the extract, a standard acute dermal toxicity testing (OECD 402) was performed. The rats were weighed before the procedure. Animals were injected with intravenous anaesthetic drug using 0.1 mL/0.1 kg of ketamine (50 mg) and xylazine (20 mg) to induce anaesthesia. After the rats were completely anaesthetized, the extracts were administered intradermally on the right foot.

Observations were performed for 14 days to monitor for any signs of a toxic reaction including weight changes, swelling, inflammation on the area of the injected foot, bleeding, and infection.

## 2.5. Antiperspirant Analysis

### 2.5.1. Samples Preparation

Appropriate amounts of 2% Tween 80, according to the weight for each rat tested, were added to each vial to achieve dose concentrations of 10, 20, 40, 80, and 160 mg/kg. For this, 2% of aluminium chloride and 2% Tween 80 in WFI were used as positive and negative controls, respectively.

### 2.5.2. Experimental Animal

A total of 48 Sprague Dawley rats (170–250 g) were obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, UKM. The rats were maintained and acclimatized under controlled temperature ( $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ), 12 h light/12 h dark conditions, for one week before the start of experiments. They were provided with water and food ad libitum. The rats were divided into 7 treatment groups ( $n = 6$ ), as the following: positive control (2% Aluminium Chloride), negative control (2% Tween 80), 10 mg/kg, 20 mg/kg, 40 mg/kg, 80 mg/kg, and 160 mg/kg samples.

### 2.5.3. Rat Foot Pad Assay

Rats were anaesthetized with intravenous injection of ketamine/xylazine. An amount of 10 mg/kg of pilocarpine HCl in water for injection (WFI) was injected subcutaneously dorsal to the scapula to induce sweating. Sweat secretion in the rats was detected using rat foot pad assays. The foot pads of the rats were cleaned with 70% ethanol to remove debris. Then, 0.1 mL hexane of *C. xanthorrhiza* was injected into interdigital palm of each rat. After that, the foot pads were swabbed with iodine (2% w/v in 95% ethanol). After the evaporation of the ethanol at room temperature, the rat foot pads were coated with castor oil containing 50% starch for 15 min. As the sweating parts of the foot pads showed coloration by starch–iodine reaction, these points on the foot pads were counted.

## 2.6. Histological Examination

The epidermis of the foot pads was dissected into pieces, fixed in 10% formalin, and processed for paraffin sectioning. Tissue sections were stained with haematoxylin and eosin and examined under an optical microscope.

## 2.7. Statistical Analysis

All data were expressed as the mean  $\pm$  standard error of mean. Values of  $p < 0.05$  were considered statistically significant. All computations were performed using GraphPad Prism<sup>®</sup> 5 for Windows software (Version 5.01, GraphPad Software, Inc., San Diego, CA, USA).

## 3. Results and Discussion

Deodorants and antiperspirants have become one of the most important products under the personal care and cosmetics category. The never-ending need of these life-style products has led to the expansion of this industry. However, association of these products with the risk of breast cancer, the rising incidence of Alzheimer's disease, and other local irritations caused by mineral pigments, colouring agents, and stabilizers used in the products may cause mild side effects such as irritation of the skin with prolonged use. Although there are contradicting opinions of the side effects, the fear of long-term side effects has influenced society to search for more natural solutions to this problem. There are several plant-based deodorants available in the market, using extracts from lime, *Melaleuca alternifolia* leaf oil, and *Eucalyptus globulus*. However, these products aim to reduce body odour without reducing excessive sweating. Hyperhidrosis is a condition in which your body sweats more than usual. While hyperhidrosis does not create odour, excessive perspiration interacts with microorganisms in your body, resulting in uncomfortable body odour. As a result, having a solution that can both reduce sweating and have an antibacterial impact is preferable.

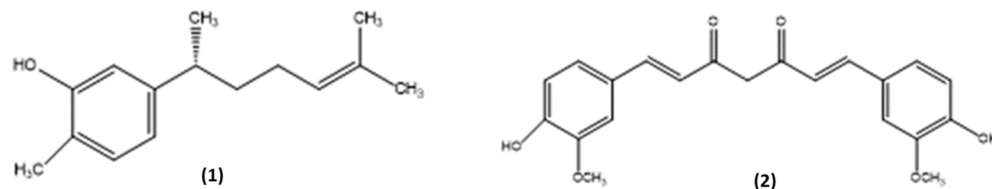
*C. xanthorrhiza* belongs to the family Zingiberaceae and has a distribution throughout southeast Asia [17]. It is a plant that originates from Indonesia, more specifically from Java Island. Most of *C. xanthorrhiza* is grown in Malaysia, Thailand, the Philippines, and Sri Lanka. It is a low-growing plant with roots or rhizomes that look like ginger. The hexane fraction of *C. xanthorrhiza* indicates the presence of terpenoids such as xanthorrhizol compounds, germacrone, zedoarol, zederone, zedoardiol, 3,4-dihydroxybisabol-1,10-diene, zedoaraldehyd,  $\alpha$ -curcumena, 8 $\beta$ -hydroxy-isogermafurenrenolide, and gelchomanolide. Meanwhile, 3-demethoxycyclocumin, 3-hydroxy-6-methylasetofenon, and gweicurculactone were found in the chloroform fraction. The fraction of ethyl acetate, on the other hand, indicates the presence of compounds 3-demetoksisikurcumin, curcumin, demetoxycumin, 1-hydroxy-1,7-bis(4-hydroxy-3-met-oxyfenil)-6-heptena-3,5-dione, and 1-(4-hydroxy-3,5-dimetoxifenil)-7-(4-hydroxy-3-methoxyfenil)-1,6-heptadina-3,5-dione. The methanol fraction also indicates the presence of sucrose, leusina, lysine, methionine, phenylalanine, triptofan, valina, alanina,  $\beta$ -glucose,  $\alpha$ -glucose, and formic acid [18].

### 3.1. Extraction and Quality Control

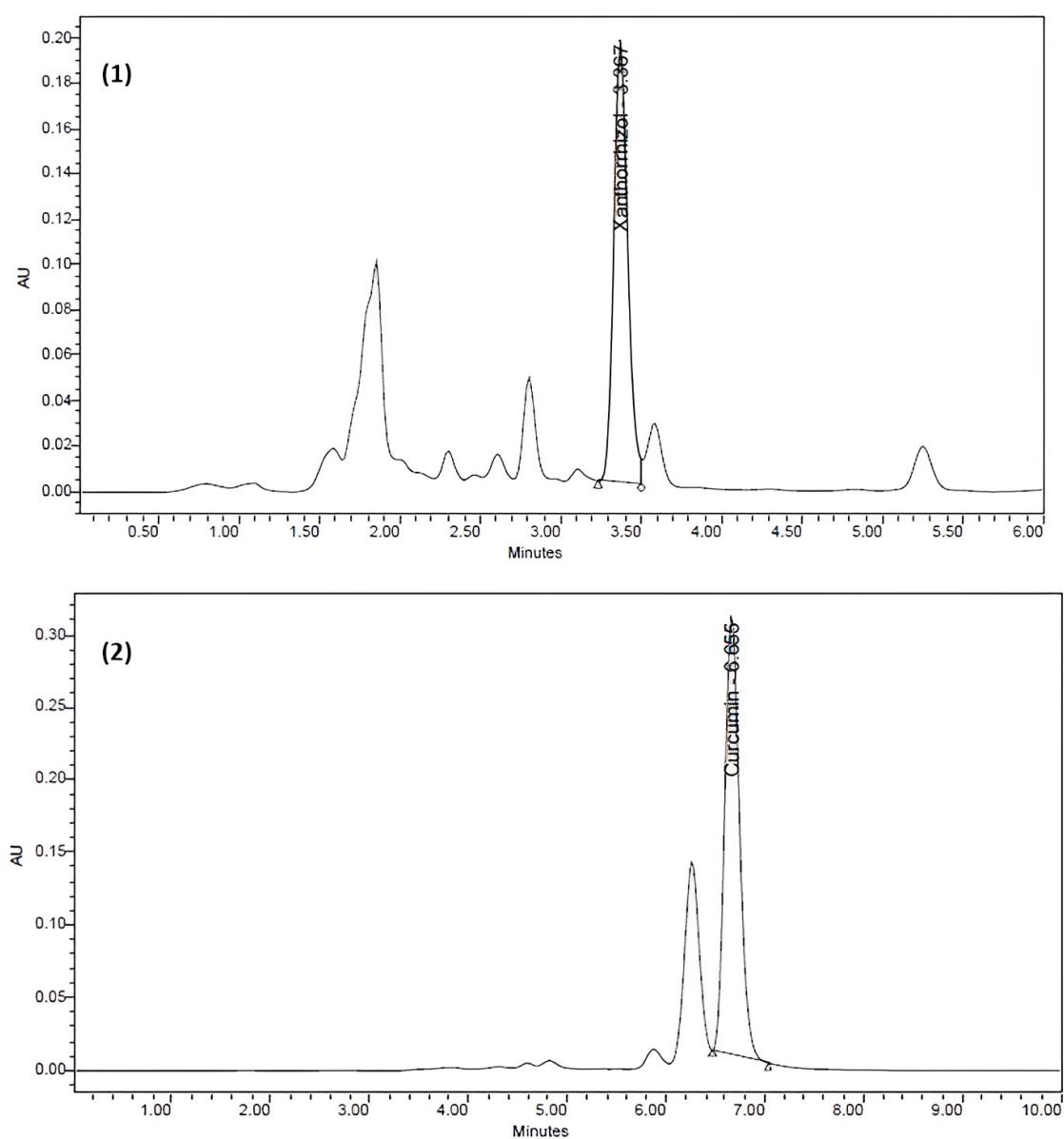
The consecutive extraction of crude ethanolic extract was conducted to ensure the optimum compound was extracted according to their polarities. The hexane fraction of *C. xanthorrhiza* gave the highest yields of extraction, which was 58.3% compared to ethyl acetate extract (20.63%) and ethanol (16.12%). High yield percentage of the hexane fraction indicated that *C. xanthorrhiza* contains high non-polar compounds. This was in agreement with a study from Devaraj et al. 2014, which stated that the fraction of hexane produced the highest percentage yield, followed by the fraction of ethyl acetate [19]. Terpenoid such as xanthorrhizol, germacrone, zedoarol, zedoaraldehyd, gweicurculactone, gelchomanolide, 8 $\beta$ -hydroxy-isogermafurenolide,  $\alpha$ -curcumene, 3,4-dihydroxybisabol-1,10-diene, and zederone are reported to be the dominant compounds in the hexane fraction [18].

HPLC was used to evaluate the quality of the extract prior to the antiperspirant and antimicrobial assays in order to exclude variations in bioactivity caused by inconsistency

of herbal extract preparations. For quality control purposes, two standard compounds, namely curcuma and xanthorrhizol (Xnt), were selected as markers for quantification of the extract (Figure 1). Xnt is a unique marker for *C. xanthorrhiza* as it can distinguish this plant from other curcuma species [20]. Figure 2 shows the chromatogram of both compounds.



**Figure 1.** The selected chemical markers in hexane extract of *C. Xanthorrhiza*; xanthorrhizol (1) and curcumin (2).



**Figure 2.** Identification of the chemical markers in *C.xanthorrhiza* hexane fraction; (1) xanthorrhizol, (2) Curcumin.



Three batches of the extract preparations were quantified under optimum running conditions. The retention times (RT) of Xnt and curcumin were found to be 3.4 min at 230 nm and 6.6 min at 425 nm, respectively, based on RT comparison of the standard compound. The quantity of the selected markers is listed in Table 1.

**Table 1.** Quantification of *C. xanthorrhiza* chemical markers in 500 µg/mL hexane extract.

Chemical Markers	Concentration µg/mL (%)	Retention Time (min)
Xanthorrhizol	218.02 (43.6)	3.4
Curcumin	7.53 (1.51)	6.6

The HPLC method was validated for its accuracy, specificity, linearity, and LOD-LOQ parameters. The linearity and accuracy of the method was established via the calibration curve of both reference standards (Table 2). Parameters such as the correlation coefficient, y-intercept, slope of regression line, and residual sum of squares were evaluated. The calibration curve was established in triplicate. The regression equation was calculated based on the mean response vs. the concentration where the regression value ( $R^2 \geq 0.99$ ) indicated the method has good linearity. The precision, repeatability, and reproducibility of the method were assessed by using intra- and interday experiments (Tables 3 and 4). The interassay RSD values were less than 5% for both standard compounds, indicating good quality of the extract and consistency between different batches.

**Table 2.** Linearity, limit of detection (LOD), and limit of quantification (LOQ) of marker compounds.

Chemical Markers	Concentration Range (µg/mL)	Linear Equation	LOD (µg/mL)	LOQ (µg/mL)
Xanthorrhizol	6.25–100	$y = 6634.9x - 4395$	3.70787	11.23599
Curcumin	6.25–100	$y = 92867x - 405163$	11.13907	33.75475

**Table 3.** Intra-assay precision of marker compounds.

Chemical Marker	Concentration (µg/mL)	% RSD Retention Time	% RSD Peak Area
Xanthorrhizol	100	0.302	0.80
Curcumin	100	0.094	1.55

**Table 4.** Intermediate precision of marker compounds.

Chemical Marker	Concentration (µg/mL)	% RSD Retention Time	% RSD Peak Area
Xanthorrhizol	50	0.181	0.52
	25	0.284	1.26
	12.5	0.330	0.41
Curcumin	100	0.181	0.52
	25	0.259	1.93
	12.5	0.061	0.23

### 3.2. In Vitro Antimicrobial Assay

Humans have two different types of sweat glands: the eccrine glands and the apocrine glands. Eccrine sweat glands are found in large numbers on the soles of the feet, the palms, the forehead, the cheeks, and in the armpits. These glands produce large volumes of watery, usually odourless sweat. Apocrine glands are different. They are found in the armpits and genital region. They produce a thick, viscous, usually invisible fluid. When this fluid comes in contact with bacteria on the skin's surface, it produces a characteristic potent smell.

The varied environment of the skin results in locally dense or sparse populations, with Gram-positive organisms (e.g., staphylococci, micrococci, diphtheroids) usually predominating. Gram-negative bacteria, on the other hand, make up a small proportion

of the skin flora. Most of them are not typical resident skin microflora but may cause cutaneous infections.

*S. epidermidis*, *S. haemolyticus*, *C. jeikeium*, and *C. tuberculostearicum* were selected in this study due to their important role in the production of unpleasant body odour in the presence of sweat [21–23]. In general, the staphylococcus species isolated from the human body contribute to the production of short-chain volatile fatty acids such as isovaleric acid from L-leucine, a branched aliphatic amino acid. Isovaleric acid has a foul odour associated with acid odour from body odour [24]. *S. epidermidis* emits an unpleasant odour on the axilla of children and adults, along the metabolism pathways of pyruvates and branched-chain amino acids. The enzymes from the epidermidis are involved in various metabolic flows leading to the production of sour odours associated with acetic acid and isovaleric acid [22]. 3M3SH, 3M2H, and HMHA were identified as the odourant compounds responsible for the unpleasant smell of the axilla in adults [24]. *S. haemolyticus* isolated from the axillary region is able to metabolize Cys-Gly-3M3SH into 3M3SH [23]. *S. haemolyticus* was confirmed as a producer of sulphur and acid odours that make the body smell less pleasant when compared to *S. epidermidis*.

*C. jeikeium*, a lipophylic bacteria, is another microorganism that produces odour in the axilla area and indicates high C-S β-lyase activity [25]. *C. jeikeium* is a species of lipophylic bacteria, which means its growth is enhanced by the presence of lipids. Analysis of the genome sequence indicates that this characteristic is most likely to occur from the absence of fatty acid sintase, which causes lipids to be an important nutrient to this species for its growth.

*C. tuberculostearicum* is the dominant species of Corynebacterium on the skin surface of the axilla and produces sulphur-smelling compounds [26]. Individuals with high unpleasant body odour intensity have high types of *C. tuberculostearicum* bacteria in the axilla section.

The antibacterial assay was conducted against selected skin microbiota identified to cause body odour. Table 5 shows the zones of growth inhibition on the tested bacteria strains where both crude ethanol extract and hexane fraction of the extract showed positive inhibitory effects.

**Table 5.** Average diameter of the inhibition zone (mm) of *C. xanthorrhiza* crude ethanol extract and its fraction extracts of different polarity at 50 mg/mL against body odour-causing bacteria.

Samples (50 mg/mL)	Inhibitory Zone (mm) <i>n</i> = 3			
	<i>Staphylococcus epidermidis</i>	<i>Corynebacterium jeikeium</i>	<i>Corynebacterium tuberculostearicum</i>	<i>Staphylococcus haemolyticus</i>
<i>C. xanthorrhiza</i> (ethanol extract)	17.67 ± 0.58 * <sup>‡</sup>	15.33 ± 0.58 * <sup>‡</sup>	20.67 ± 0.58 * <sup>‡</sup>	14.67 ± 0.58 * <sup>‡</sup>
Hexane fraction	25.33 ± 1.0 * <sup>‡</sup>	20.33 ± 1.15 * <sup>‡</sup>	26.67 ± 0.58 * <sup>‡</sup>	16.33 ± 0.58 * <sup>‡</sup>
Ethyl acetate fraction	0.0 ± 0.0 <sup>‡</sup>	0.0 ± 0.0 <sup>‡</sup>	0.0 ± 0.0 <sup>‡</sup>	0.0 ± 0.0 <sup>‡</sup>
Ethanol fraction	0.0 ± 0.0 <sup>‡</sup>	0.0 ± 0.0 <sup>‡</sup>	0.0 ± 0.0 <sup>‡</sup>	0.0 ± 0.0 <sup>‡</sup>
Positive control (1 mg/mL Gentamicin)	30.33 ± 0.49 *	26.67 ± 0.83 *	32.41 ± 0.51 *	29.42 ± 0.67 *
Negative control (5% DMSO)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

The value is expressed as the average ± standard deviation (SD) (*n* = 3); Analysis was conducted with one-way ANOVA, followed by Dunnet test; \* *p* < 0.0001 compared to negative control, <sup>‡</sup> *p* < 0.0001 compared to positive control.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were further determined in Tables 6 and 7, indicating that the hexane fraction of *C. xanthorrhiza* was the most promising inhibitor as it can inhibit bacterial growth of up to 83.5% compared to the positive control. Corynebacterium spp. have been reported to produce the most unpleasant and strong malodours (James et al. 2004), and the potent inhibition of *C. jeikeium* (zone of inhibition = 20.33 ± 1.15 mm and MIC = 0.049 mg/mL) and *C. tuberculostearicum* (zone of inhibition = 26.67 ± 0.58 mm and MIC = 0.049 mg/mL) by the hexane fraction of *C. xanthorrhiza* was particularly encouraging



**Table 6.** The MIC values of tested samples and controls against *S. epidermidis*, *C. jeikeium*, *C. tuberculostearicum*, and *S. haemolyticus*.

Plant Extracts	Minimum Inhibitory Concentration (µg/mL) <i>n</i> = 3			
	<i>Staphylococcus epidermidis</i>	<i>Corynebacterium jeikeium</i>	<i>Corynebacterium tuberculostearicum</i>	<i>Staphylococcus haemolyticus</i>
<i>C. xanthorrhiza</i> (ethanol extract)	0.391 ± 0.0 **	0.195 ± 0.0	0.391 ± 0.0 *	0.391 ± 0.0 **
Hexane fraction	0.024 ± 0.0	0.049 ± 0.0	0.049 ± 0.0	0.098 ± 0.0
Ethyl acetate fraction	ND	ND	ND	ND
Ethanol fraction	ND	ND	ND	ND
Positive control (Gentamicin)	0.007 ± 0.0	0.017 ± 0.0	0.063 ± 0.0	0.031 ± 0.0
Negative control (5% DMSO)	ND	ND	ND	ND

The value is expressed as the average ± standard deviation (SD) (*n* = 3); Analysis was conducted with one-way ANOVA, followed by Dunnet test; \* *p* < 0.05 \*\* *p* < 0.01 as compared to positive control. ND: Non-detected.

**Table 7.** The MBC values of tested samples and controls against *S. epidermidis*, *C. jeikeium*, *C. tuberculostearicum*, and *S. haemolyticus*.

Plant Extracts	Minimum B Concentration (µg/mL) <i>n</i> = 3			
	<i>Staphylococcus epidermidis</i>	<i>Corynebacterium jeikeium</i>	<i>Corynebacterium tuberculostearicum</i>	<i>Staphylococcus haemolyticus</i>
<i>C. xanthorrhiza</i> (ethanol extract)	0.391 (+) **	0.195 (+)	0.391 (+) *	0.391 (+) *
Hexane fraction	0.024 (+)	0.049 (+)	0.049 (+)	0.098 (+)
Ethyl acetate fraction	ND	ND	ND	ND
Ethanol fraction	ND	ND	ND	ND
Positive control (Gentamicin)	0.017 (+)	0.031 (+)	0.125 (+)	0.031 (+)
Negative control (5% DMSO)	ND	ND	ND	ND

The value is expressed as the average ± standard deviation (SD) (*n* = 3); Analysis was conducted with one-way ANOVA, followed by Dunnet test; \* *p* < 0.05 \*\* *p* < 0.01 as compared to positive control. ND: Non-detected; (+): bactericidal.

Both ethanol and the hexane fraction of the extract showed significant inhibition of all bacteria compared to the negative control (*p* < 0.0001). The hexane extract has proven to have a better activity than the ethanol extract. Besides that, the antimicrobial activity of the hexane extract presented with low MIC and MBC values and bactericidal activity for all tested bacteria. Previous study has shown good antimicrobial activity of the extracts against different types of bacteria including *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella thypi*, and *Vibrio cholerae* [27].

The antibacterial effect of *C. xanthorrhiza* was due to the secondary metabolites in the rhizome, which contains active compounds in the form of essential oils, curcumin, alkaloids, flavonoids, terpenoids, and phenols that are the result of plant secondary metabolics. Curcumin and xanthorrhizol have a broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. Curcumin works by preventing cell proliferation and altering bacterial cell dimming and making the movement in and out of the material into the cell become uncontrolled [28]. Substances in cells such as organic ions, enzymes, amino acids, and nutrients can leave those bacterial cells. When enzymes leave bacterial cells along with substances such as water and nutrients, there will be metabolic inhibition and a resulting decrease in adenosine triphosphate (ATP) necessary for growth and proliferation of bacterial cells. Finally, inhibition of bacterial cell growth occurs and causes death in the bacterial cells.

### 3.3. Toxicity Analysis

Toxicity tests were conducted according to the OECD 402 guidelines with a small modification, where samples were injected on the intradermal part of the rat's foot. In vivo toxicity tests conducted for 14 days on Sprague Dawley rats showed that the hexane fraction of *C. xanthorrhiza* was safe to use. There were no signs of toxicity on the rats tested for extract concentration up to 2000 mg/kg. No swelling, rashes, bleeding, or infections occurred in the observed injection area. Furthermore, this test confirmed the appropriateness of handling and sample injection techniques via the intradermal route into the rat foot pads. These results are confirmed with normal behavioural patterns, clinical signs of normal animals, and histopathological analysis on important organs that did not show toxic effects [29].

### 3.4. Rat Foot Pad Analysis

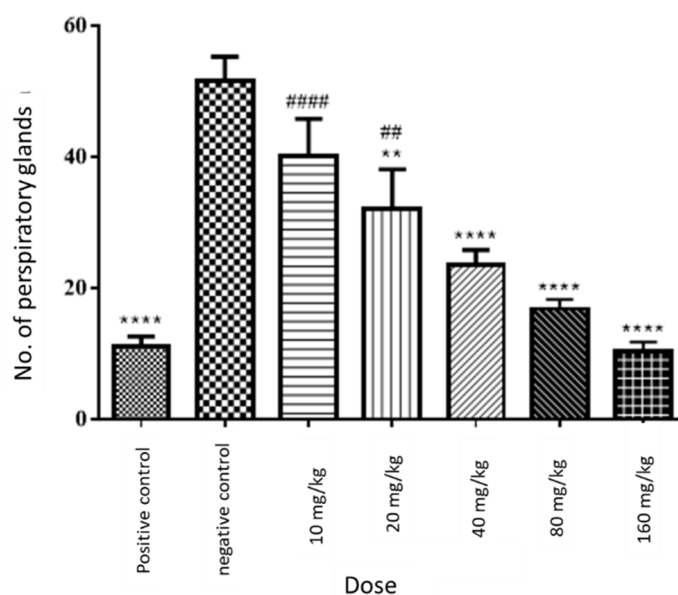
Rat foot pads as a model for an antiperspirant assay was first introduced by Lansdown (1973) [30]. In a more recent study, Wang et al. (2016) investigated the effect of Erxian decoction and Fructus schisandrae chinensis (11 g/kg) on sweat reduction among menopausal mice of Sprague Dawley species [31]. Our research adapted the rat foot pad method used with some modifications. In this investigation, rats were administered with the hexane fraction of *C. xanthorrhiza* at concentrations of 10, 20, 40, 80, and 160 mg/kg, as well as 2% AICI as a positive control, to promote sweating. The negative control group did not receive any treatment (only given an injection of pilocarpine HCl).

Sweat secretion on the rat foot pads was determined by the iodine–starch reaction that produced blue-black spots with the presence of sweat. Figure 3 shows the appearance of black spots on rats' foot pads after administration of the lowest and highest concentrations of extracts in comparison to the controls after fifteen minutes. The appearance of these spots represents the number of active sweat glands. The total number of active sweat glands for all extract concentrations is shown in Figure 4. It was observed that with the increase in the dose of the hexane extract of *C. xanthorrhiza*, the amount of sweat decreased.



**Figure 3.** The appearance of black spots on the rat food pad 15 min post administration of (A) positive standard, (B) negative standard, (C) control, (D) 10 mg/kg extract, and (E) 160 mg/kg extract.

The reduction percentage in comparison with the negative control is tabulated in Table 8. Rat sweat secretion was reduced by all concentrations of the hexane fraction of *C. xanthorrhiza*. A concentration of 40 mg/kg resulted in a significant decrease in the number of active sweat glands when compared to the negative control ( $p = 0.001$ ), with a 53% decrease in the percentage of active sweat glands. Concentrations of 80 mg/kg and 160 mg/kg reduced the number of sweat glands by 66.34% and 79.34%, respectively, when compared to the negative control ( $p = 0.0001$ ).



**Figure 4.** Antiperspirant effect of hexane extract of *C. xanthorrhiza* ( $n = 6$ , mean  $\pm$  standard error of mean), one-way ANOVA, followed by Dunnet test; \*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$  as compared to negative control; ##  $p < 0.01$ , ###  $p < 0.0001$  compared to positive control.

**Table 8.** Percentage of active sweat gland reduction compared to negative control.

Sample Group (mg/kg)	Percentage of Active Sweat Gland Reduction (%)
Positive control	77.66
10	22.33
20	37.54
40	53
80	66.34
160	79.34

A reduction in axillary sweating of 20% or more is required in treatment with topical antiperspirant products to demonstrate that the product is effective [32]. When compared to the negative control, treatment with a concentration of up to 160 mg/kg reduced sweat secretion by 79.34% in this study. This demonstrates the ability of the *C. xanthorrhiza* hexane fraction to effectively reduce sweating rate.

In this work, pilocarpine HCl, a cholinergic agent that binds muscarinic receptors to eccrine sweat glands and induces the production of sweat, was used to induce the sweating in rats. Rats do not sweat in response to elevation of core body temperature to at least 41 °C, while humans sweat to thermoregulate their body temperature. The sweat glands of rodents, such as rats, are only present on their foot pads [31].

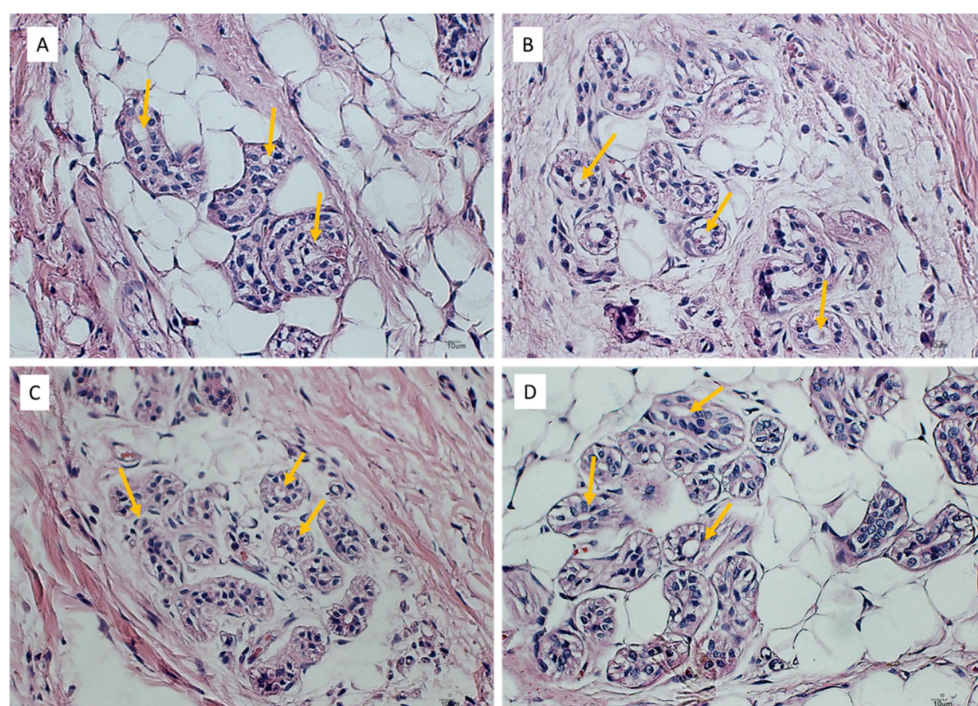
AICI at 2% was used as a positive control as 1–5% aluminium compounds are commonly used in topical antiperspirants on the market today [33]. Aluminium salts have an astringent effect on sweat pores, causing them to shrink and further preventing sweat from reaching the skin's surface [32]. Individuals suffering from hyperhidrosis, on the other hand, require a higher concentration to treat this problem of excessive sweating more effectively; a 15% solution of AICI or higher is usually used, with about a week of use required to maintain the results [34].

All dosages of the hexane fraction of *C. xanthorrhiza* were able to inhibit sweat secretion, especially the doses of 40, 80, and 160 mg/kg, which significantly decreased the sweat secretion ( $p < 0.05$ ) compared to the negative control. Figure 4 shows that with the increase in the dose of the hexane extract of *C. xanthorrhiza*, the sweat will decrease (based on the number of the sweat glands). Table 8 shows a comparable value of the percentage of active

sweat gland reduction between the positive control and sample extract at 160 mg/kg, which indicates the antiperspirant activity of *C. xanthorrhiza* was particularly encouraging.

### 3.5. Histology Analysis

The sweat glands on the plantar legs of the rats are located in the section of the hypodermis fat cell group where there is irregular dermis connective tissue and many blood vessels around the secretion area. Controlled rats not receiving pilocarpine HCl injections indicated normal eccrine glands on the rats' foot pads (Figure 5A). The sweat glands are surrounded by dark-coloured secretory epithelium cells with pink layer myoepithelial cells that have the property to shrink and expand.



**Figure 5.** Secretory portions of rat plantar sweat glands in foot pads (indicated by solid yellow arrows) (A) normal control; (B) negative control (injected with pilocarpine); (C) positive control (2% AlCl); (D) Hexane extract of *C. xanthorrhiza* (40 mg/kg). (H&E stain,  $\times 400$ .)

The observation of the lumen size is indicated with the arrows. The normal lumen size of the glands' secretory units is small (Figure 5A). For the negative control group which was injected with pilocarpine HCl, the lumen size of the eccrine sweat gland expanded from large to moderate size (Figure 5B). The positive control group which was treated with 2% AlCl post pilocarpine injection showed a smaller size of the lumen compared to the negative control (Figure 5C). The sample group which was treated with the hexane extract of *C. xanthorrhiza* presented small- to medium-sized lumens, comparable to the positive control (Figure 5D).

### 4. Conclusions

Research on antiperspirant activity from plant extracts has not been fully explored yet. Many naturally sourced antiperspirants and deodorant products available in the market are not fully backed by fundamental and clinical data. The authors believe that this provides first-time data on the potential of the hexane fraction from *C. xanthorrhiza* extract as an antiperspirant and antibacterial agent against skin microbiota causing body odour.

For the evaluation of antibacterial activity, the hexane fraction of *C. xanthorrhiza* showed good activity in inhibiting bacteria identified to cause body odour; *S. epidermidis*



ATCC 12228, *S. haemolyticus* ATCC 29970, *C. jeikeum* ATCC 43734, and *C. tuberculo-stearicum* ATCC 35692.

The same extract showed promising antiperspirant activity where concentrations of 10–160 mg/kg reduced the number of active glands up to 79.34% when compared with the negative control. Observation of the histological changes of the rat foot pad showed the size of the lumen in the secretory unit of the eccrine gland decreasing after administration of the extract.

Standardization and quality control of the extract were conducted using RP-HPLC based on two main markers of the plants, xanthorrhizol (43.6%) and curcumin (1.51%), in 500 µg/mL hexane fraction. In addition, the extract was found to be safe up to 2000 mg/kg.

The data obtained from this study allow the production of topical antiperspirant products based on medicinal plants. Active compounds from this extract may also be tested in other hyperhidrosis treatments such as iontophoresis.

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**Data Availability Statement:** Supporting data can be obtained from Universiti Kebangsaan Malaysia Thesis Repository (Nurliyana Athirah; Master Thesis, 2021).

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