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Antimicrobial activities of ethanol and aqueous extracts of *Piper betle* seed and *Citrus aurantifolia* leaf against axillary bacteria causing body odour

Tukur S. T.[1]*, Zainab Ibrahim Bala[2] and Ibrahim Hamisu Abdul[3]

[1]Department of Science Laboratory Technology, Federal Polytechnic, Kabo, Kano State, Nigeria.
[2]Department of Life Sciences, Kano State Polytechnic, Kano, Nigeria.
[3]Department of Pharmaceutical Technology, Federal Polytechnic, Kabo, Kano State, Nigeria.

*Corresponding author. Email: sanitajo88@gmail.com/sanitajo@fedpolykabo.edu.ng, Tel: +234 7031957141.

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ABSTRACT: This research aimed to investigate the antimicrobial activities of ethanol and aqueous extracts of *Piper betle* seed and *Citrus aurantifolia* leaf against axillary bacteria that cause body odour. The objectives were to identify the bacteria from human auxiliary sweat with odour problem and to test the efficacy of *Piper betle* (Ashanti pepper) seed and *Citrus aurantifolia* leaf extracts against the bacteria isolated. Axilla sweat samples were taken from 16 males with odour problems, using a sterile cotton swab to swab both the left and right axilla skin. Afterwards, the specimen was cultured on MacConkey. All of the bacterial pure cultures were identified using Gram staining. Several biochemical tests, including oxidase test, catalase test, coagulase test and Triple sugar iron test (TSI) were carried out. Reference microorganisms (*Staphylococcus aureus, Enterobacteriaceae, Klebsiella, Salmonella, Shigella and Escherichia coli*) were identified using the same methods. From the study, there is no increased effect from the combination of these extracts against bacteria. *Piper betle* seeds and *Citrus aurantifolia* leaves had sufficient potential to warrant further examination and development as a new antibacterial agent. Infusion *of Piper betle* seeds and *Citrus aurantifolia* leaves has antibacterial activity as antiseptic preparations that can inhibit the growth of some test bacteria. It can be recommended that further research should be carried out to identify and isolate bioactive substances which contribute to antibacterial activity from these plants.

Keywords: *Citrus aurantifolia,* Gram staining**,** microorganisms, microbiota, *Piper betle.*

INTRODUCTION

Sweating is a normal physiological process in our body to regulate our temperature. Excessive sweating occurs when we are exposed to hot temperatures, during heavy exercise or in stressful and anxiety conditions. Sweat is an odourless fluid secreted by sweat/sudoriferous glands that are distributed in our skin. There are two types of sweat gland: the eccrine gland and the apocrine gland. The eccrine glands are distributed over most of our body and are responsible for the watery secretions responsible for regulating body temperature. These glands perform the important functions of thermoregulation and excretion. Body odour, also known as bromhidrosis, osmidrosis, or bromidrosis, is a typical occurrence in post-pubertal people. Bromhidrosis may occasionally progress into pathology if it is especially severe or if it adversely affects the lives of those who are affected. Bromhidrosis is a chronic illness in which the skin exudes an excessive amount of odour, typically an offensive one. Apocrine gland secretion has a significant role in determining bromhidrosis, which can significantly lower a person's quality of life (Nathaniel *et al*., 2018).

Unpleasant underarm odour is due to microbial biotransformation of odourless and sterile secretions into volatile odour molecules caused by enzymes released by

the microbiome in sweat glands, pores, and hair follicles (Natsch and Emter, 2020). In the armpit, there are glandular fields with discrete bacterial colonies, which ideally live in a sweaty and humid environment where oily and odourless fluids are the main source of nourishment (Callewaert *et al.*, 2017). The bacterial flora of the axilla proved to be a stable mixture of Gram-positive bacteria, including *Micrococcus luteus* (*M. luteus*), *Bacillus subtilis* (*B. subtilis*), *Staphylococcus epidermidis* (*S. epidermidis*), and *Corynebacterium xerosis* (*C. xerosis*) (Al-Talib *et al*., 2016). Odourless secretions are secreted by eccrine, sebaceous, and apocrine glands to regulate body temperature (Bovell, 2015). In contrast to primary bacterial catabolism, the bacterial flora of the axilla undergoes processes such as steroid biotransformation of long-chain fatty acids to release short volatile branched fatty acids (Minhas,2018). People tend to use various topical deodorants and antiperspirants to minimise the spread of underarm odour. Deodorants work by lowering the bacterial count and masking the bad odour with fragrance molecules such as ethanol or an antibacterial agent that kills the bacteria (Roberts, 2018). On the other hand, antiperspirants contain aluminium chloride and reduce moisture in the armpits by temporarily clogging the skin pores, leading to the sweat glands (Pariser and Ballard, 2014).

Citrus aurantifolia is a perennial evergreen tree that can grow to a height of 3–5 m. Stem: irregularly slender, branched and possesses short and stiff, sharp spines or thorns 1 cm or less. Leaves: alternate, elliptical to oval, 4.5–6.5 cm long, and 2.5–4.5 cm wide with small rounded teeth around the edge. Petioles are 1–2 cm long and narrowly winged. Flowers: short and axillary racemes, bearing few flowers which are white and fragrant. Petals are 5, oblong, and 10–12 mm long. Fruits: green, round, 3–5 cm in diameter, it is yellow when ripe. All citrus fruits present the same anatomical structures. Flavedo is the external part of the fruit and has a lot of flavonoids, as its name suggests. The outer cell wall is composed of wax and cutin for prevention of water loss from the fruit; albedo is the white spongy portion, below the flavedo layer; carpal membranes or septum presenting around 8–11 glandular segments, usually aligned and situated around the soft central core; juice sacs are yellow-green pulp vesicles; and seeds are small, plump, ovoid, pale, and smooth with white embryo (Zia *et al*., 2013).

Therefore, the following objectives are put forward to help in achieving the aim, these are:

1. To isolate the bacteria from human auxiliary sweat with odour problems.
2. To identify the bacteria from human auxiliary sweat with odour problems.
3. To carry out the phytochemical screening of the extracts using ethanolic and aqueous solutions.
4. To test the efficacy of *Piper betle* (Ashanti pepper) and *Citrus aurantifolia* leaf extracts against bacteria isolated.

MATERIALS AND METHODS

Materials used in this research were: Nutrient Agar, Muller Hillton Agar, Gram staining reagents (Lugol's iodine, carbon fuchsin and ethanol), Swap stick, Peptone broth media, Biochemical test reagent, Ethanol (96%), DSMO solution, Glassware: Common Glass Laboratory Equipment, Filter Paper 6 mm In Diameter, Autoclave, Incubator and Oven, Water bath And Light Microscope, Petri Dish, Fuel Paper, Cotton Wool masting Tape, Plant samples, *Piper betle seed. leaf* and Citrus *aurantifolia* (L).

Sample size

To calculate the total sample, we use the formula:

$$\mathrm{n} = \frac{z^2 P(1-P)}{d^2}$$

Where: n = minimum sample size required, z = Standard deviation set at 95% i.e. 1.965, P = Expected prevalence i.e. 9% = 0.09, d = Desired precision i.e. 10% = 0.1.

$$\mathrm{n} = \frac{(1.965)^2 \; x \; 0.09(1-0.09)}{(0.5)^2}$$

$$\mathrm{n} = \frac{3.842 \; x \; 0.09(0.91)}{0.25}$$

$$\mathrm{n} = \frac{0.3146598}{0.25}$$

$$\mathrm{n} = 15.587$$

n ≈ 16 samples

Sample collection

Axilla sweat samples were taken from 16 males with odour problems, using the swab method. Fresh leaves of *Piper betle* seed and *Citrus aurantifolia* were collected from Yankura market, Sabon Gari axis of Fagge Local Government of Kano State. The leaf of Citrus *aurantifolia* was washed with running water, air-dried, put into a drying cabinet at 40°C, and powdered mechanically. The seed of *Piper betle* was further dried at room temperature and ground into powder form. Both were stored for further analysis in the course of this research.

Bacterial isolation from axilla sweat

Axilla sweat samples were taken from 16 males with odour problems, using a sterile cotton swab to swab both the left and right axilla skin. Afterwards, the specimen was

cultured on MacConkey agar (MA) overnight at 37°C and identified using catalase and coagulase tests. After confirming the specimen, microbial culture was taken using a sterile culture and dipped into a test tube containing 5 ml of PBS. Incubated at 37°C for two hours until the suspension turbidity is appropriate to McFarland's 0.5 turbidity standard.

Bacterial identification

All of the bacterial pure cultures were identified using Gram staining. Several biochemical tests, including oxidase test, catalase test, coagulase test and Triple sugar iron test (TSI) were carried out. Reference microorganisms (*S. aureus, Enterobacteriaceae, Klebsiella, Salmonella, Shigella* and *E. coli*) were identified using the same methods as described by Cappuccino and Natalie (2019).

Phytochemical screening of the plant samples

Phytochemical components of the leaves of *C. aurantifolia* and the seeds of *P. betel* were screened by using the standard methods. The compounds screened were alkaloids, flavonoids, cardiac glycosides, tannins, phenolics, steroids, saponins, phytosterols, quinones and coumarins using standard known methods as described by Obidoa *et al*. (2009).

Test for alkaloids

A quantity (0.2 g) of the sample was boiled with 5 ml of 2% HCl on a steam bath. The mixture was filtered, and a 1 ml portion of the filtrate was measured into four test tubes. Each of the 1 ml filtrates was treated with 2 drops of the following reagents (Obidoa *et al*., 2009).

Test for flavonoids

A quantity (0.2 g) of each of the extracts was heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture was filtered differently, and the filtrates were used for the following tests: Using Ammonium Test: A quantity (4 ml) of each of the filtrates was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow colouration was observed at the ammonia layer, which indicates the presence of flavonoids (Obidoa *et al*., 2009).

Test for glycosides

Dilute sulphuric acid (5 ml) was added to 0.1 g each of the extracts in a test tube and boiled for 15 minutes in a water bath. It was then cooled and neutralized with 20% potassium hydroxide solution. A mixture, 10 ml of equal parts of Fehling's solution A and B were added and boiled for 5 minutes. A denser red precipitate indicates the presence of a glycoside (Obidoa *et al*., 2009).

Test for steroids

A quantity (9 ml) of ethanol was added to 1 g each of the extracts, and refluxed for a few minutes and filtered. Each of the filtrates was concentrated to 2.5 ml in a boiling water bath. Distilled water, 5 ml, was added to each of the concentrated solutions, and each of the mixtures was allowed to stand for 1 hour, and the waxy matter was filtered off. Each of the filtrates was extracted with 2.5 ml of chloroform using a separating funnel. To 0.5 ml of each of the chloroform extracts in a test tube were carefully added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface shows the presence of steroids (Obidoa *et al*., 2009).

Test for terpenoids

To another 0.5 ml, each of the chloroform extracts was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on a water bath. Grey indicates the presence of terpenoids (Obidoa *et al*., 2009).

Test for saponins

A quantity (0.1 g) of each of the extracts aqueous and n-hexane) were boiled with 5 ml of distilled water for 5 minutes. The mixture was filtered while still hot, and the filtrates were used for the following test:

Emulsion Test: A quantity (1 ml) of each of the filtrates was added drops of olive oil. The mixture was added to another two drops of olive oil. The mixture was shaken and observed for the formation of an emulsion (Obidoa *et al*., 2009).

Test for tannins

A quantity (2 g) of each of the extracts (n-hexane and water) was boiled with 5 ml of 45% ethanol for 5 minutes. Each of the mixtures was cooled and filtered. The different filtrates were subjected to the following tests.

Ferric Chloride Test: A quantity (1 ml) of each of the filtrates was diluted with distilled water, and 2 drops of ferric chloride were added. A transient greenish to black colour indicates the presence of tannins (Obidoa *et al*., 2009).

Preparation of plant extracts

Aqueous extract

Thirty grams of leaf powder was suspended in 300 ml of

distilled water. Aqueous extraction was done at 70˚C for 30 minutes. After that, the extracts were filtered by using Whatman filter paper No.1. Then, the extracts were evaporated at 45˚C to form a paste, and after that, transferred into sterile bottles and refrigerated at 4˚C until use.

Ethanolic extract

Organic solvent extraction of the *Citrus aurantifolia* leaves powder (10.0mg/ml) was carried out by using ethanol (80%). This was done by using the Soxhlet apparatus. The extraction was carried out for 24 hours at a heating temperature that kept the solvent at 50-60˚C until a colourless and clear solvent appeared in the extracting chamber. After that, the extract was dried in an electric oven at a temperature of 40- 45˚C until a dry extract was obtained. The final extract was kept frozen at –18˚C until use.

Determination of antibacterial activity

The extracts of *Piper betle* seed and *C. aurantifolia* leaf were tested against bacterial isolates from axillary patients by first marking the Petri dish containing Mueller-Hinton agar (MHA) with the desired extract concentrations and creating streaks of bacterial isolates on top of the MHA. Next, six holes with a diameter of 6 mm each were punched aseptically with a sterile borer and 25 µL of the anti-microbial agent or extract solution of 10.0 mg/ml at desired concentrations of 0.10 mg/ml, 0.25 mg/ml and 0.20 mg/ml were introduced into each well then left to incubate at 37°C and evaluated the next day to see the clear zone formation surrounding the well as described by Tebabal Kebede (2021). The clear zone diameter measurement was done using a calliper. Each treatment was repeated three times.

Statistical analysis

A database was developed to store qualitative and quantitative data from the cross-sectional study using Microsoft Excel 2010 spreadsheet. STATA version 11 was used to compute descriptive statistics of variables collected during the study. Overall bacterial load was calculated using descriptive statistics of the sample through frequencies and cross-tabulations.

RESULTS

Phytochemical analysis of lime leaf, Ashanti seed ethanolic and aqueous extracts

The phytochemical screening results of lime leaf show that all the secondary metabolites examined were present except steroids, alkaloids, and quinone in ethanolic extracts. The results also show that all the secondary metabolites screened were present except saponins, flavonoids, phenols, steroids, alkaloids and quinone in aqueous extracts (Tables 1 and 2).

The phytochemical screening results of Ashanti seed show that all the secondary metabolites were present except steroids, alkaloids, quinone and phytosterol in ethanolic extracts. The results also show that all the secondary metabolites were present except tannins, phenols, flavonoids, alkaloids and phytosterol in aqueous extracts (Tables 3 and 4).

Phytochemical screening results showed that both extracts contained alkaloids, flavonoids, tannins, phenolics, cardiac glycosides, steroids, and quinones (Tables 1 and 2). the results also show that antibacterial activity from Citrus *aurantifolia* and *Piper. betle* leaves of ethanolic and aqueous extract against body odor (bromhidrosis) bacteria (Tables 3 and 4).

Zone of inhibition of the lemon leaf plant aqueous and ethanolic extract against some isolated organisms

The results show that the MIC for *Escherichia coli*, *Enterobacteriaceae*, *S. aureus, Klebsiella and S. typhi* is 25 mg/ml. It gives the same concentration of 25 mg/ml, which demonstrates a pre-determined concentration in CFU/ml when compared to the MIC dilution volume in MBC using ethanolic and aqueous extract (Table 5).

Isolation and identification of Bacteria from human axillae with odour problem

Different type of bacteria was isolated using MacConkey agar in the laboratory are identified based on their morphological characteristics. Biochemical test of bacteria isolated from axillary sweat samples of males experiencing body odour was conducted as presented in Tables 6, 7 and 8.

Table 7 shows the results of the morphological test conducted to identify the bacteria isolated from the sample of the sweat obtained from the bodies of males with odour. Sample A was observed to be pinkish, indicating *S. aureus,* as sample B shows pinkish dry and flat, indicating *Enterobacteriaceae* and also, sample C shows pink mucor, indicating *Klebsiella*, while sample D was colourless and flat, indicating that *Salmonella* and *Shigella* are present. As sample E shows a dry pink colour, indicating the presence of *E. coli.* Table 8 shows that all the samples from A to E are all positive to the biochemical test using catalase, coagulase, oxidase and Tripple sugar iron test to test for the bacterial from the axilla sweat, as some of the sample shows the samples were yellow, red slanted with gas present and acidic, except sample C which shows no acid present.

Sample A in Gram staining shows *Streptobacillus* in

Table 1. Result of the secondary metabolites of lime leaf ethanolic extract.

S/N	Phytochemicals	Observation	Test methods	Results (ve)
1	Saponins	Formation of foams	Frothing test	+
2	Cardiac glycoside	Ring purple color	Kellakiuiani test	+
3	Steroids	No reaction	Salkowoski test	-
4	Flavonoids	Yellow coloration	NaOH test	+
5	Tannins	Blue-black precipitate	Ferric chloride	+
6	Alkaloids	No reaction	Mayer's test	-
7	Phytosterol	Golden yellow colour	Salkowoski test	+
8	Phenols	White Precipitate	Lead acetate test	+
9	Quinone	No reaction	KOH test	-
10	Cumarins	Formation of yellow	NaOH test	+

Key: + = positive, - = negative.

Table 2. Result of the secondary metabolites of lime leaf inference aqueous extract.

S/N	Phytochemicals	Observation	Test methods	Results (ve)
1	Saponins	A stable froth was observed	Frothing	-
2	Cardiac glycoside	Dense red precipitate	Kellakiiani test	+
3	Steroids	Reddish-brown interface	Salkowoski test	+
4	Flavonoids	Yellow coloration	NaOH test	-
5	Tannins	A greenish color that changed to red observed.	Ferric chloride	+
6	Alkanoids	Creamy-white colored precipitate	Mayer's test	-
7	Phytosterol	Grayish colour	Salkowoski test	+
8	Phenols	Clear color	Lead acetate test	-
9	Quinone	No reaction	KOH test	-
10	Cumarins	Light brown	NaOH test	-

Key: + = positive, - = negative.

Table 3. Result of the secondary metabolites of Ashanti seed ethanolic extract.

S/N	Phytochemicals	Observation	Test methods	Results
1	Saponins	Formation of foams	Frothing test	+
2	Steroids	A reddish-brown color	Salkowoski test	+
3	Flavonoids	Yellow coloration	NaOH test	+
4	Tannins	Blue-black precipitate	Ferric chloride	+
5	Cardiac glycoside	Purple ring color	Kellakiuiani test	+
6	Alkaloids	No reaction	Mayer's test	-
7	Phytosterol	No reaction	Salkowoski test	-
8	Phenols	Creamy Precipitate	Lead acetate test	+
9	Quinone	Reddish color	KOH test	-
10	Cumarins	Formation of yellow	NaOH test	-

Key: + = positive, - = negative.

clusters with a pinkish colour. Sample B in Gram staining shows Streptococcus in clusters with a purple colour. Also, Sample C in Gram staining shows *Streptococcus* in the cluster with a purple colour. As Sample D in Gram staining shows cocci in clusters with a purple colour. While Sample E in Gram staining shows cocci with a purple colour.

Sample F was observed to be pinkish, indicating *S. aureus,* as sample G shows pinkish dry and flat, indicating *Enterobacteriaceae* and also, sample H shows pink mucor, indicating *Klebsiella*, while sample I was colourless flat, indicating that *Salmonella* and *Shigella* are present. As sample J shows dry pink colour, indicating the presence of *E. coli.*

Sample K was observed to be pinkish, indicating *S. aureus,* as sample L shows pinkish dry and flat, indicating

Table 4. Result of the secondary metabolites of Ashanti seed inference aqueous extract.

S/N	Phytochemicals	Observation	Test methods	Results
1	Saponins	Formation of foams	Frothing test	+
2	Steroids	A reddish-brown color	Salkowoski test	+
3	Flavonoids	Yellow coloration	NaOH test	-
4	Tannins	Blue-black precipitate	Ferric chloride	-
5	Cardiac glycoside	Purple ring color	Kellakiuiani test	+
6	Alkanoids	No reaction	Mayer's test	-
7	Phytosterol	No reaction	Salkowoski test	-
8	Phenols	Creamy Precipitate	Lead acetate test	-
9	Quinone	Reddish color	KOH test	+
10	Cumarins	Formation of yellow	NaOH test	+

Key: + = positive, - = negative.

Table 5. MIC and MBC in mg/ml of ethanolic extracts of lemon lea extract against different test organisms.

Isolate	MIC Vol	MBC Vol
E. coli	25mg/ml (dilution)	25mg/ml
S. aureus	25mg/ml (dilution)	25mg/ml
S. typhi	25mg/ml (dilution)	25mg/ml
Klebsiella	25mg/ml (dilution)	25mg/ml
Enterobacteriaceae	25mg/ml (dilution)	25mg/ml

Table 6. Bacteria isolated from axilla sweat on MacConkey agar.

Sample	Morphological	isolates
A	Pinkish mucric	*S. aureus*
B	Pinkish dry and flat	*Enterobacteriace*
C	Pink mucord	*Klebsella*
D	Colourless flat	*Salmonella /Shigella*
E	Dry pink	*E. coli*
F	Pinkish mucric	*S. aureus*
G	Pinkish dry and flat	*Enterobacteriace*
H	Pink mucord	*Klebsella*
I	Colourless flat	*Salmonella /Shigella*
J	Dry pink	*S. epidermides*
K	Pinkish mucric	*S. aureus*
L	Pinkish dry and flat	*Enterobacteriace*
M	Pink mucord	*S. haemolyticus*
N	Colourless flat	*Salmonella /Shigella*
O	Dry pink	*S. epidermides*
P	Pinkish mucric	*S. aureus*

Key: A – P = Numbers of samples 16 males.

Enterobacteriaceae and also, sample M shows pink mucor, indicating *Klebsiella*, while sample N was colourless flat, indicating that *Salmonella* and *Shigella* are present. As sample O shows dry pink colour, indicating the presence of *E. coli.* As sample P shows a greenish colour, indicating the presence of Bacilli.

To test the efficacy of *Piper betle* (Ashanti pepper) and *Citrus aurantifolia leaf* extracts against bacteria isolated

Two plant species were investigated to evaluate their antibacterial activity against some bacteria, including

Table 7. Morphological characteristics of the bacteria isolated from axilla sweat on MacConkey agar.

Samples	Gram staining	Color
A	Strepto baccitic in clusters	Pink
B	Steph Cocci in cluster	Purple
C	Steph Cocci in chain	Purple
D	Cocci in cluster	Purple
E	Cocci	Purple
F	Strepto baccitic in clusters	Pink
G	Steph Cocci in cluster	Purple
H	Steph Cocci in chain	Purple
I	Cocci in cluster	Purple
J	Cocci	Purple
K	Strepto baccitic in clusters	Pink
L	Steph Cocci in cluster	Purple
M	Steph Cocci in chain	Purple
N	Cocci in cluster	Purple
O	Cocci	Purple
P	Baccilli	Greenish

Key A – P = Numbers of samples 16 males.

Table 8. Biochemical test of bacterial isolated from axilla sweat.

Samples	Biochemical tests			TSI
	CA	COA	OX	
A	+	+	+	Red slant. Yellow booth, gas present with acid present
B	+	+	+	Red slant. Yellow booth, gas present with acid present
C	+	+	+	Red slant. Yellow booth, gas present, no acid
D	+	+	+	Red slant. Yellow booth, gas present with acid present
E	+	+	+	Red slant. Yellow booth, gas present with acid present
F	+	+	+	Red slant. Yellow booth, gas present with acid present
G	+	+	+	Red slant. Yellow booth, gas present with acid present
H	+	+	+	Red slant. Yellow booth, gas present, no acid
I	+	+	+	Red slant. Yellow booth, gas present with acid present
J	+	+	+	Red slant. Yellow booth, gas present with acid present
K	+	+	+	Red slant. Yellow booth, gas present with acid present
L	+	+	+	Red slant. Yellow booth, gas present with acid present
M	+	+	+	Red slant. Yellow booth, gas present, no acid
N	+	+	+	Red slant. Yellow booth, gas present with acid present
O	+	+	+	Red slant. Yellow booth, gas present with acid present
P	+	+	+	Red slant. Yellow booth, gas present with acid present

Key: CA = Catalase, COA = Coagulase, OX = Oxidase, TSI – Triple Sugar Iron, + = Positive, - = Negative.

strains of Gram-positive (*S*. *aureus*) and strains of Gram-negative (*E. coli*), using the disc diffusion method. Evaluation of the antibacterial activity of these plant extracts was recorded in Tables 9 and 10. The results revealed that all plant extracts were potentially effective in suppressing microbial growth of bacteria with variable potency. *Ashanti pepper* was the most effective extract retarding microbial growth of all tested pathogenic bacteria at the highest concentration of 100 ug/ml in aqueous form while ethanolic extract of *Ashanti paper* was effective only against *S. aureus*. Other plant extracts showed variable antimicrobial activity against body odour bacterial strains. *Citrus aurantifolia* leaf exhibited inhibitory effect against other pathogenic strains (Klebsiella, *S. aureus*, *E. coli,* Enterobacteriaceae). Table 10 shows that the MBC of ethanolic for *Escherichia coli, Klebsiella, Enterobacteriaceae, S. aureus and S. typhi* are all positive at a concentration of 25 mg/ml.

Table 9. Sensitivity test of ethanolic and aqueous extracts of lemon leaf.

Extracts	Conc.	Z – I	Z – I
	100µg/ml	23.00±0.13^{b}	
Lemon leaf (Aq)	50µg/ml	12.00±0.23^{b}	30.00±0.23
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	22.00±0.13^{ba}	
Lemon leaf (Eth)	50µg/ml	13.00±0.14^{b}	19.00±0.22
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	24.00±0.13^{b}	
Lemon leaf (Aq)	50µg/ml	23.00±0.13^{ab}	24.00±0.13
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	20.00±0.13^{ab}	
Lemon leaf (Eth)	50µg/ml	17.00±0.1^{ac}	25.00±0.13
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	21.00±0.13	
Lemon leaf (Aq)	50µg/ml	18.00±0.17	27.00±0.22
	25µg/ml	0.00±0.13^{b}	
	100µg/ml	24.00±0.13^{b}	
Lemon leaf (Eth)	50µg/ml	23.00±0.13^{ab}	24.00±0.13
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	22.00±0.13^{ba}	
Lemon leaf (Aq)	50µg/ml	13.00±0.14^{b}	19.00±0.22
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	22.00±0.13^{ba}	
Lemon leaf (Eth)	50µg/ml	13.00±0.14^{b}	30.00±0.23
	25µg/ml	0.00±0.19^{c}	

Statistically significant difference by paired t-test, $p<0.05$. Key: Values are Means±Standard deviations of triplicate determinations. Values in the same column sharing the same letters are not significantly different at 5 % level. (Odebunmi *et al*., 2009).

DISCUSSION

Deodorants and antiperspirants make up one of the largest segments in the health and beauty industry, with the global market anticipated to reach USD 92,707 million by 2024 (Zion Market Research, 2019). 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 (Taucher *et al.*, 2017). Numerous studies supporting and discrediting this claim have been published (National Cancer Institute Factsheet, 2017). Besides, alarming concerns on the association of other main active ingredients used in deodorants and antiperspirants, like aluminium 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 two medicinal plants traditionally used to reduce body odour against some specific species of skin microbiota responsible for causing body odour, intending to produce a clinically safe and effective ingredient for deodorant and antiperspirant.

The use of some aromatic plants as traditional medicine to face body odour problem could be a simple and good alternative solution besides the use of commercial/ chemical deodorant-antiperspirant products. It is cheaper, easier and safer than the use of chemical ingredients contained in such products. Darbre *et al.* (2004) stated that the paraben compound, that usually contained in deodorant-antiperspirant products, could cause irritation on axilla skin in long-term application and may induce mamae cancer

Table 10. Sensitivity test of ethanolic and aqueous extracts of Ashanti leaf.

Extracts	Conc.	Z – I	Z – I
	100µg/ml	23.00±0.13^{b}	
Ashanti leaf (Aq,)	50µg/ml	12.00±0.23^{b}	30.00±0.23
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	24.55±0.13^{ba}	
Ashanti leaf (Eth,)	50µg/ml	16.11±0.14^{b}	20.32±0.22
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	24.00±0.13^{b}	
Ashanti leaf (Aq,)	50µg/ml	23.00±0.13^{ab}	24.00±0.13
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	20.00±0.13^{ab}	
Ashanti leaf (Eth,)	50µg/ml	17.00±0.1^{ac}	25.00±0.13
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	21.00±0.13	
Ashanti leaf (Aq,)	50µg/ml	18.00±0.17	27.00±0.22
	25µg/ml	0.00±0.13^{b}	
	100µg/ml	21.57±0.13^{b}	
Ashanti leaf (Eth,)	50µg/ml	19.16±0.13^{ab}	22.00±0.13
	25µg/ml	18.98±0.19^{c}	
	100µg/ml	22.00±0.13^{ba}	
Ashanti leaf (Aq,)	50µg/ml	13.00±0.14^{b}	19.00±0.22
	25µg/ml	0.00±0.19^{c}	
	100µg/ml	21.60±0.13^{ba}	
Ashanti leaf (Eth,)	50µg/ml	21.13±0.14^{b}	27.69±0.23
	25µg/ml	13.61±0.19^{c}	

Statistically significant difference by paired t-test, $p<0.05$. Key: Values are Means±Standard deviations of triplicate determinations. Values in the same column sharing the same letters are not significantly different at 5 % level. (Odebunmi *et al*., 2009).

after a long period. However, these findings remain debatable among researchers.

Human underarm odour is a frustrating and bothersome health problem and has led to a bad perception of individuals who sweat uncontrollably. It is not only unpleasant, but it can also cause psychological disorders if left untreated (Callewaert *et al.*, 2017). In some cases, body odour may develop due to poor hygiene and poor metabolism associated with the daily diet.

The result of the efficacy test of *Piper betle* (Ashanti pepper) and *Citrus aurantifolia leaf* extracts against bacteria was in agreement with Hermawan et al. in his study, influence of leaf extract of sirih (*Piper betle* L.) to growth *Staphylococcus aureus* and *Escherichia coli* with disk diffusion method, which showed significant results in all concentrations of *Piper betle* L. extract analysed in his study (Hermawan *et al.,* 2008). In this study, the concentrations analysed were added to see whether or not *Piper betle* L. leaf extract exhibits an antibacterial ability at concentrations lower than 25 µg/ml.

The antibacterial potential of betel leaves (*Piper betle* L.) is the result of the presence of various activated compounds. The compounds of *Piper betle* L. leaf extract have been confirmed by a phytochemical analysis, identification and quantification research conducted by Syahidah *et al.* (2107). The study shows the presence of contained alkaloids, flavonoids, tannins, phenolics, cardiac glycoside, steroids, and quinones.

Conclusions

From the result of this study, it is revealed that *Piper betle* and *Citrus aurantifolia* leaf extract have great potential as

a natural source of therapeutic agents for bacterial infection, especially for Bromhidrosis. Nevertheless, the leaf extracts of *Piper betle* and *Citrus aurantifolia* need to be further investigated before they can be used as an alternative therapy for bacterial Bromhidrosis and other bacterial pathogens, such as in *vivo* testing and toxicity testing in animal models. *Piper betle* and *Citrus aurantifolia* inhibited the growth of three strains of skin microbiota, causing body odour and thus indicated promising medicinal plants in the development of natural deodorant and antiperspirant. *Piper betle* and *Citrus aurantifolia* had sufficient potential to warrant further examination and development as a new antibacterial agent. Study to isolate other axilla pathogens than this one is highly encouraged, and further research should be carried out to identify and isolate bioactive substances which contribute to antibacterial activity from these plants.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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