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Antibacterial Screening of Two Indigenous Nigerian Spices; *Piper guineense* and *Xylopia aethiopica*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OCA and NRI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AAU and DRT managed the analyses of the study and prepared the final manuscript. Authors OCA and AAU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Leaf and fruit extracts of *Piper guineense and Xylopia aethiopica* were respectively evaluated for their antibacterial activities against two (2) organisms; *Staphylococcus aureus and Pseudomonas aeruginosa.* The antibacterial activity was measured by agar well diffusion method using 8 mm diameter cork borer. All the extracts except hot aqueous extract of *P. guineense* leaves showed antibacterial activity against the test bacterial isolates. Cold aqueous extract of *X. aethiopica* did not exhibit antibacterial activity against *P. aeruginosa,* also hot aqueous extract of *X. aethiopica* did not exhibit antibacterial activity against *P. aeruginosa.* The ethanolic extracts of *P. guineense* showed inhibitory activity against the two test bacterial isolates with zone diameter of inhibition that ranged between 12.5 mm to 28.0 mm, while *X. aethiopica* showed activity with zone diameter of inhibition that ranged between 11.5 mm to 20.0 mm. The cold aqueous extracts of *P. guineense* showed antibacterial activity against both test bacterial isolates with zone diameter of inhibition that ranged between 12.0 mm to 25.0 mm, while *X. aethiopica* showed activity only against *S. aureus* with zone

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of inhibition ranged between 9.5 mm to 19.0 mm. The hot aqueous extracts of *P. guineense* showed no antibacterial activity against the test organisms, while that of *X. aethiopica* showed activity only against *S. aureus* with zone of inhibition ranged between 9.0 mm to 25.0 mm. Amoxicillin and Ciprofloxacin antibiotics were used as positive controls, while the extracting solvents were used as negative controls. The phytochemical screening revealed the presence of saponins, flavonoids, and tannins and the absence of alkaloids in both spices studied. The MIC result revealed variability in the inhibitory concentration of each extract for both organisms tested. All active extracts of *Piper guineense* where found to possess an MIC of 3.12 mg/ml, while the MIC range for all the active extracts of *Xylopia aethiopica* ranged between 3.12 mg/ml to 12.5 mg/ml. Based on this finding, these extracts show promise and form a primary platform for further phytochemical and pharmacological studies for use as alternative medicine.

Keywords: Antibacterial; phytochemical; inhibitory; agar well; pharmacological.

1. INTRODUCTION

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods [1]. Spices include leaves (bay, mint, rosemary, coriander, laurel, and oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, and black pepper), stems (coriander, cinnamon), rhizomes (ginger, turmeric) and other plant parts [2].

Spices are used as condiments and ingredients in foods. In Nigeria, some are used for the preparation of certain type of soups which may be taken hot or cold especially during the cold, rainy seasons and also recommended for fast relief of ailments such as malaria fever [3]. Various studies have shown that apart from using spices for flavouring and seasoning foods, beverages and medicines, they have several other wide applications in local treatment and management of many diseases [4]. Spices which include plant materials of medicinal importance have been used for treatment of human ailments as far back as prehistoric times [5].

Although pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [6]. Such a fact is cause for concern, because of the number of patients in the hospitals who have suppressed immunity, and also due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality [7].

The problem of microbial resistance is growing and the outlook for use of antimicrobial drugs in the future is still uncertain. Therefore, actions need be taken to reduce this problem, for example, to control the use of antibiotics, develop research to better understand the genetic mechanisms of resistance, and to engage on further studies to develop new drugs, either synthetic or natural with no or minimal side effects. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient [8].

Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [9,10] and there is a considerable global interest in tapping the accumulated knowledge of traditional medicine thus researches are being carried out in many countries with the aim of increasing the use of traditional medicine to the welfare of the human population [11].

Therefore there is urgent and continuous need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [12]. Hence more studies pertaining the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes.

In this study the effect of aqueous and ethanolic extracts of the spices *Piper guineense* and *Xylopia aethiopica* were tested against one gram positive organism; *Staphylococcus aureus* and one gram negative organism; *Pseudomonas aeruginosa.*

Piper guineense and *Xylopia aethiopica* present such potential of high medicinal value. The plants are used in Nigeria traditionally as condiments and for treatment of various ailments such as rheumatism, bronchitis, dysentery and stomach

ache. The fruit of *Xylopia aethiopica* are used as a cough medicine, as a carminative and, to some extent as a purgative. *Piper guineense* is used as a carminative, especially for griping conditions. In eastern Nigeria there is an age-long practice to mix dried seeds of *Xylopia aethiopica* and *Piper guineense* in soups made for nursing mothers as it is believed to have germicidal activities in the womb.

2. MATERIALS AND METHODS

The research basically involved laboratory experiments which were conducted in accordance with standard laboratory procedures.

2.1 Study Site

The study area of this research was University of Abuja Main Campus, Airport Road in F.C.T Abuja, North Central Zone of Nigeria.

2.2 Sample Collection

Xylopia aethiopica fruits and *Piper guineense* leaves were purchased from spice vendors in Gwagwalada market, Gwagwalada Area Council, Abuja in May, 2014. The plants were identified and authenticated with Voucher Number: sp. 312 and sp. 313 respectively in the taxonomy unit of the Department of Biological Sciences, University of Abuja, Abuja, Nigeria.

2.2.1 Test organisms

The test organisms were obtained from the Microbiology Laboratory of University of Abuja Teaching Hospital, (UATH). Cultures of these test organisms were purified and confirmed by repeated sub-culturing and microscopy and maintained on nutrient agar slants at 4ºC. The test organisms include:-

Gram-positive: *Staphylococcus aureus*
Gram-negative: *Pseudomonas aeruginosa*

2.2.2 Sterilization and media preparation

All apparatus used were sterilized properly using the autoclave at 121℃ for (15 minutes). The work bench was disinfected with 70% ethanol before and after use.

All media used for the analysis were prepared according to manufacturers' instructions. The media used include: Nutrient agar grams/liter (Park Scientific Limited), Mannitol Salt Agar grams/liter (Med Micro), Mueller Hinton Agar grams/liter (Liofilchem Limited), Nutrient Broth grams/liter (Park Scientific Limited).

2.2.3 Confirmation of purity and vaibility of test organisms

The test organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa)* were sub-cultured repeatedly by quadrant streaking and observed for growth after 24 hours at 37℃. Yellowish colonies on mannitol salt agar and greenish colonies on the nutrient agar were observed and were finally sub-cultured onto nutrient agar.

2.2.3.1 Gram staining

Different kinds of bacteria react differently to Gram stain because of structural differences in their cell wall. Therefore, Gram staining technique was performed on the various bacterial types in order to separate the bacteria into two large groups, Gram positive and Gram negative and further tests conducted to confirm the isolates used in this study.

The sub cultured organisms were gram stained for identification and observation using the procedure described by Madigan et al. [13].

2.2.3.2 Microscopy

The slides were observed by the light microscope using the x100 objective lens with oil immersion. *Staphylococcus aureus* was observed and characterized by its gram positive potential i.e. purple colored cocci shaped cells that were arranged in clusters, while the *Pseudomonas aeruginosa* was observed and characterized by its gram negative reaction i.e. pink colored rod shaped cells".

2.2.4 Preparation of plant samples for extraction

The spices were properly washed and air dried under shade to a constant weight. The dried spice samples were pulverized to fine powder using clean mortar, sieved and stored in air tight containers for further use.

2.2.4.1 Extraction

Three solvents were used in the preparation of the extracts, namely; cold deionized water, hot deionized water, and 70% ethanol. The percentage extract yield of powdered material in each solvent was calculated and recorded.

2.2.4.2 Aqueous extraction (Cold water)

The method of Al-Magboul et al. [14] as modified by Okigbo and Omodamiro [15] was used. Fifty gram each of the pulverized spices was soaked in 200 ml of cold deioned distilled water and left to stand for 24 hours. It was filtered using the Whatman no.1 filter paper and the filtrate evaporated in a water bath at 25℃ to dryness.

The % yield of the cold aqueous extract of *Piper guineense* was calculated thus;

8.9 (g) / 50 (g) x 100 =17.8%

The % yield of the cold aqueous extract of *Xylopia aethiopica* was calculated thus;

8.0 (g) ÷ 50 (g) x 100 = 16.0%

2.2.4.3 Aqueous extraction (Hot water)

The method of Okigbo and Mmeka [16] was used and it involved soaking 12 g each of the pulverized spices in 100 ml of hot water and boiling for 30 minutes in a 500 ml beaker. It was filtered using the Whatman no.1 filter paper and the filtrates evaporated in a water bath at 25℃ to dryness.

The %yield of the hot aqueous extract of *Piper guineense* was calculated thus;

1.7 (g) / 12.0 (g) x 100 =14.1%

For the % yield of the ethanolic extract of *Xylopia aethiopica* was calculated thus;

1.5 (g) / 12.0 (g) x 100 = 12.5%

2.2.4.4 Organic solvent extraction using ethanol

The method of Nweze et al. [17] was used. Fifty gram each of the pulverized spices was soaked in 450 ml of 70% ethanol solvent and left to stand for 48 hours. It was filtered using the Whatman no.1 filter paper and the filtrates evaporated at room temperature to dryness.

For the % yield of the ethanolic extract of *Piper guineense* was calculated thus;

10.5 (g) / 50 (g) x 100 = 21.0%

For the % yield of the ethanolic extract of *Xylopia aethiopica* was calculated thus;

6.9 (g) / 50 (g) x 100 = 13.8%

2.2.5 Phytochemical analysis of plant extracts

Qualitative phytochemical analyses were carried out according to standard procedures as described by Harborne [18]; Sofowora [3]; Trease and Evans [19]. The samples were screened for saponins, tannins, balsams, flavonoids, alkaloids, sesquiterpenes, terpenoids, phlobatanins, reducing sugar and steroids.

2.2.5.1 Screening of the plant extracts for antibacterial effect

Extracts were sterilized by membrane filtration using the 0.45 µm membrane filter (Sterlitech) and reconstituted to concentrations of 100 mg/ml, 50 mg/ml 25 mg/ml and 12.5 mg/ml respectively.

2.2.6 Preparation of bacterial culture

The stock culture of each of the bacteria used was sub cultured on nutrient agar by streaking an inoculum from the slant culture and incubated at 37℃ for 24 hours. The 24 hour culture was emulsified in 3 ml of sterile water to obtain a suspension.

2.2.6.1 Agar well diffusion assay

The antibacterial activity of the six (6) extracts (Ethanolic, Hot aqueous and cold aqueous) of two plants against two bacterial isolates was evaluated using the modified agar well diffusion method of Perez et al. [20] and Hammer et al. [21]. The poured Mueller Hinton agar plates were solidified and seeded with the suspensions of test organisms (in duplicates) and allowed to stand for 15 minutes. Wells of 8 mm diameter size were made with a sterile cork borer into the agar plates containing the bacteria inoculums. Using a sterile Pasteur's pipette 0.2 ml of each concentration of plant extracts was introduced into each of the wells of inoculated plates. Ethanol and sterile deionized water (hot and cold) were used as negative controls. Simultaneously, Amoxicillin (for *Staphylococcus aureus*) and Ciprofloxacin (for *Pseudomonas aeruginosa*) were positive controls. The dilution medium for the positive controls was sterile distilled water. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extracts into the agar [22]. After incubation for 24 hours at 37℃, the plates were observed for clear zones. Each clear zone was measured in millimeters using a transparent meter rule and expressed as the zone diameter

of inhibition produced by the plant extracts. The mean values were calculated and antibacterial activity was recorded if the zone diameter of inhibition was greater than 8 mm [21].

2.2.6.2 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the extracts that exhibited considerable activity was evaluated using the tube dilution method as described by Isu and Onyeagba [23]. An aliquot of 0.1 ml of the already prepared bacterial suspension of each organism to be tested was added to a series of 8 sterile test tubes of nutrient broth containing two fold (2:2) dilution of the extracts at concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.12 mg/ml, 1.56 mg/ml and 0.78 mg/ml. Control tubes containing only the plant extracts and the growth medium, the growth medium of each of the bacterial suspension, and nutrient broth alone were also prepared. All tubes were incubated at 37℃ for 24 h. The lowest concentration that did not permit any visible bacterial growth (turbidity) when compared with the control tubes was considered as the minimum inhibitory concentration.

2.2.6.3 Determination of minimum bactericidal concentration (MBC)

The contents of all tubes that showed no turbidity (visible growth) were inoculated on Nutrient agar plates and incubated at 37℃ for 24 h [24]. The minimum bactericidal concentration was considered as the lowest concentration that produced no single bacterial colony after incubation.

2.3 Statistical Analysis

All statistical analyses of data from various treatments were carried out using analysis of variance (ANOVA) test using factorial experiment. Detailed analysis (arithmetic mean, standard deviation, relative standard deviation and correlation coefficient) were performed on the derived data using standard methods. The software SPSS (Statistical Package for Social Science) was employed for the statistical analysis.

3. RESULTS

3.1 Qualitative Phytochemical Components of *Piper guineense* Leaf Extracts and *Xylopia aethiopica*

The results of the phytochemical screening of the spice extracts are presented on "Table 1", it was observed that Saponins, Terpenoids and Tannins were extracted by almost all the solvents. Furthermore, ethanol extracted all the phytochemicals tested except reducing sugar, Terpenoids and alkaloids from *Xylopia aethiopica* and glycosides, balsams, alkaloids, sesquiterpenes, phlobatannins and steroids from *Piper guineense*. Generally, more phytochemicals were extracted from *X. aethiopica*.

Table 1. Qualitative Phytochemical components of *Piper guineense* leaf extracts and *Xylopia aethiopica* fruit extracts

Phytochemical	Hot aqueous *Piper guineense*	Cold aqueous *Piper guineense*	Ethanol *Piper guineense*	Hot aqueous *Xylopia aethiopica*	Cold aqueous *Xylopia aethiopica*	Ethanol *Xylopia aethiopica*
Saponins	+	+	+	+	+	+
Glycosides	_	_	_	+	_	+
Tannins	_	+	+	+	+	+
Balsams	_	_	_	+	+	+
Flavonoids	_	+	+	_	+	+
Alkaloids	_	_	_	_	_	_
Sesquiterpenes	_	_	_	+	+	+
Terpenoids	+	+	+	+	+	_
Phlobatannins	_	_	_	+	+	+
Reducing sugar	+	+	+	_	_	_
steroids	_	_	_	+	_	+

Key: (+) = Present; (-) = Absent

3.2 Antibacterial Activity of the Ethanolic Extracts of *Piper guineense* and *Xylopia aethiopica*

From "Fig. 1" it was observed that the test organism *Staphylococcus aureus* was susceptible to the ethanol extract of both *Piper guineense* and *Xylopia aethiopica* at varying degree, although the effect of *X. aethiopica* was not as much as that of *P. guineense*. Also the test organism was not susceptible to the negative control (ethanol) but was susceptible to the positive control (Amoxicillin), furthermore *Pseudomonas aeruginosa* was susceptible to the ethanol extract of both *Piper guineense* and *Xylopia aethiopica* at varying degree, although the effect of *P. guineense* was slightly higher than that of *X. aethiopica*. Also the test organism was not susceptible to the negative control (ethanol) but was susceptible to the positive control (Ciprofloxacin).

3.3 Antibacterial Activity of the Cold Aqueous Extracts of *Piper guineense* and *Xylopia aethiopica*

From "Fig. 2" it was observed that the test organism *Staphylococcus aureus* was susceptible to the cold water extracts of both *Piper guineense* and *Xylopia aethiopica* at varying degree, although the effect of *X. aethiopica* was not as much as that of *P. guineense*. Also the test organisms were not susceptible to the negative control (cold water) but were susceptible to the positive controls (Amoxicillin), further *Pseudomonas aeruginosa* was susceptible to the cold water extract of *Piper guineense* but was resistant to the extract of *X. aethiopica*. Also the test organism was not susceptible to the negative control (cold water) but was susceptible to the positive control (Amoxicillin).

3.4 Antibacterial Activity of the Hot Aqueous Extracts of *Piper guineense* and *Xylopia aethiopica*

From "Fig. 3" it was observed that the test organism *Staphylococcus aureus* was susceptible to the hot water extract of *Xylopia aethiopica*, but was resistant to the extract of *Piper guineense*. Also the test organisms were not susceptible to the negative control (hot water) but were susceptible to the positive control (Amoxicillin). Furthermore *Pseudomonas aeruginosa* was not susceptible to the hot water extract of both *Piper guineense* and *Xylopia aethiopica*. Also the test organisms were not susceptible to the negative control (hot water) but were susceptible to the positive control (Ciprofloxacin).

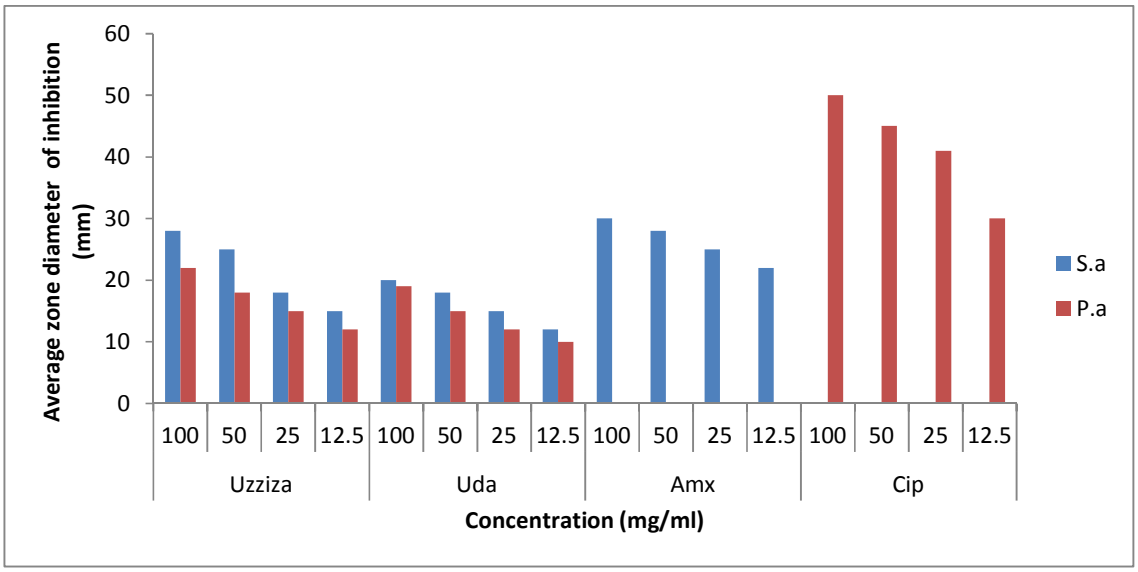

Fig. 1. Antibacterial activity of the ethanolic extracts of *Piper guineense* and *Xylopia aethiopica* and standard antibiotics against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Key: S.a= Staphylococcus aureus; P.a= Pseudomonas aeruginosa; P. g= Piper guineense; X. a= Xylopia aethiopica; Amx= Amoxicillin; Cpr= Ciprofloxacin

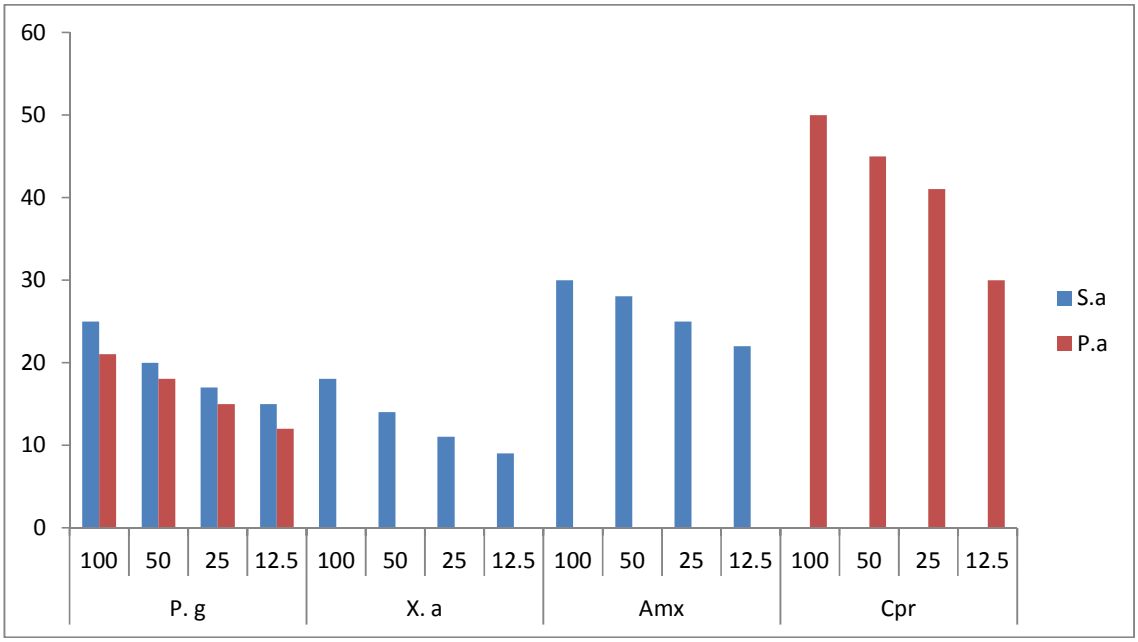

Fig. 2. Antibacterial activity of the cold aqueous extracts of *Piper guineense* and *Xylopia aethiopica* and standard antibiotics against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Key: S.a= Staphylococcus aureus; P.a= Pseudomonas aeruginosa; P. g= Piper guineense; X. a= Xylopia aethiopica; Amx= Amoxicillin; Cpr= Ciprofloxacin

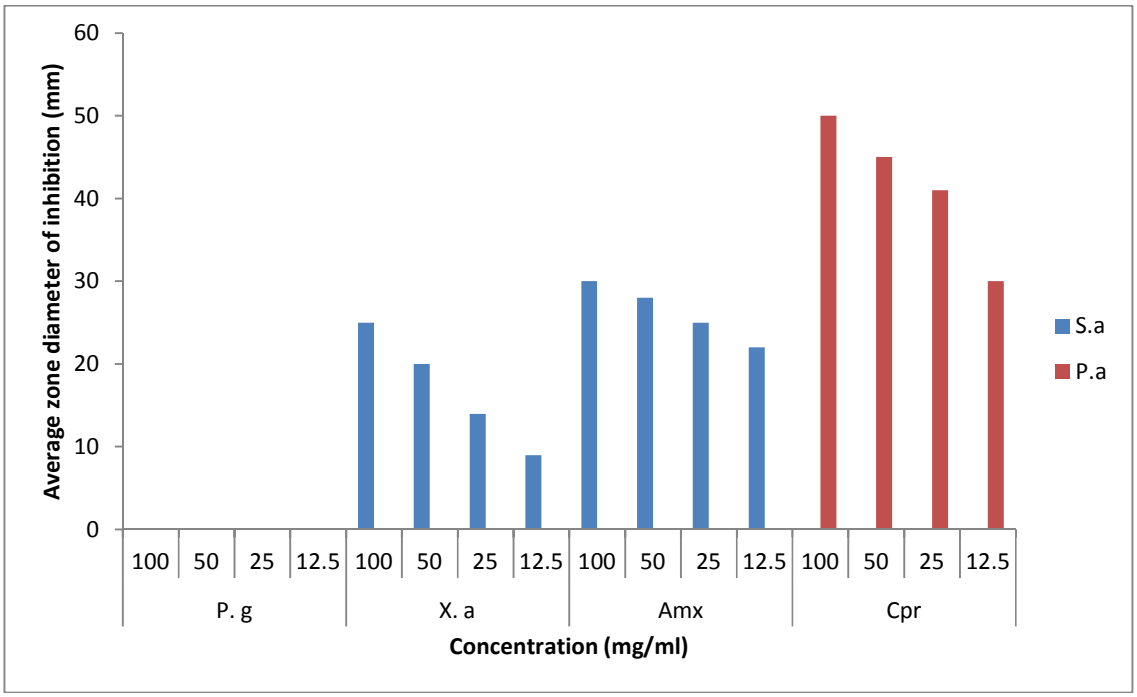

Fig. 3. Antibacterial activity of the hot aqueous extracts of *Piper guineense* and *Xylopia aethiopica* and standard antibiotics against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Key: S.a= Staphylococcus aureus; P.a= Pseudomonas aeruginosa; P. g= Piper guineense; X. a= Xylopia aethiopica; Amx= Amoxicillin; Cpr= Ciprofloxacin

3.5 Minimum Inhibitory Concentration of Ethanolic, Cold Aqueous and Hot Aqueous Extracts

The MIC result on "Table 2" revealed variability in the inhibitory concentration of each extract for both organisms tested. All active extracts of Piper *guineense* where found to possess an MIC of 3.12 mg/ml, while the MIC range for all active extracts of *Xylopia aethiopica* ranged between 3.12 mg/ml to 12.5 mg/ml.

3.6 Minimum Bactericidal Concentration of Ethanolic, Cold Aqueous and Hot Aqueous Extracts

The MBC result on "Table 3" revealed variability in the inhibitory concentration of each extract for both of the organism tested. All the active extracts of Piper *guineense* where found to possess MBC ranged between 6.25 mg/ml to 25.0mg/ml, while the MBC range for all the active extracts of *Xylopia aethiopica* ranged between 6.25 mg/ml to 12.5 mg/ml.

4. DISCUSSION

The study generated interesting data about different aspects of the extracts of *Piper guineense* leaf and *Xylopia aethiopica* fruit. The results showed that the two spice extracts significantly ($p<.05$) inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* at all concentration used with inhibition varying from one extract to another.

It was observed that the antibacterial effectiveness of these spices in culture depends on the concentration used and the solvent of extraction. Doses of 12.5-100 mg/ml of the extracts were used and from the results obtained, it can be deduced that the antimicrobial activity of the extracts increased with increasing concentrations of the extracts in all cases "Figs. 1-3". This agrees with the report of Banso et al. [25] which observed that the higher concentrations of antimicrobial substances showed appreciable growth inhibition.

Table 2. Minimum inhibitory concentration of ethanolic, cold aqueous and hot aqueous extracts of *Piper guineense* Leaves and *Xylopia aethiopica* fruits

Plant	Test organism	Solvent	Minimum inhibitory concentration (mg/ml)							
			100	50	25	12.5	6.25	3.12	1.56	0.78
P. guineense	*S. aureus*	Ethanol	---	---	---	---	---	MIC	+	+
		Cold aqueous	---	---	---	---	---	MIC	+	+
	P. aeruginosa	Ethanol	---	---	---	---	---	MIC	+	+
		Cold aqueous	---	---	---	---	---	MIC	+	+
X. aethiopica	*S. aureus*	Ethanol	---	---	---	---	---	MIC	+	+
		Cold aqueous	---	---	---	MIC	+	+	+	+
		Hot aqueous	---	---	---	MIC	+	+	+	+
	P. aeruginosa	Ethanol	---	---	---	MIC	+	+	+	+

Key: (-)= Clear (No growth); (+)=Turbid (growth); MIC =Minimum Inhibitory Concentration

Table 3. Minimum bactericidal concentration of ethanolic, cold aqueous and hot aqueous extracts of *Piper guineense* Leaves and *Xylopia aethiopica* fruits

Plant	Test organism	Solvent	Minimum bactericidal concentration (mg/ml)					
			100	50	25	12.5	6.25	3.12
P. guineense	*S. aureus*	Ethanol	----	----	----	----	MBC	+
		Cold aqueous	----	----	----	MBC	+	+
	P. aeruginosa	Ethanol	----	----	----	----	MBC	+
		Cold aqueous	----	----	MBC	+	+	+
X. aethiopica	*S. aureus*	Ethanol	----	----	----	----	MBC	+
		Cold aqueous	----	----	----	MBC	+	+
		Hot aqueous	----	----	----	MBC	+	+
	P. aeruginosa	Ethanol	----	----	----	MBC	+	+

Key: (+) growth; (-) no growth; MBC Minimum bactericidal concentration

The antibacterial activity of the various extract of the two spices was consistent against same microorganism in some cases but varied in other cases. For example, the three extracts (ethanol, cold aqueous and hot aqueous) of *Xylopia aethiopica* were consistently active against *Staphylococcus aureus* "Figs. 1-3". This probably means that the compound responsible for the antibacterial activity was present in each extract at different concentration.

The ethanol extract of the two samples had significant effect on the test organisms and provided a more consistent antibacterial activity as noted in "Fig. 1", this agrees with earlier reports by Ezeifeka et al. [26]; Allero and Afolayan [27]; Perekh and Chanda [28] who reported that ethanol extracts of spices have high potency for control of pathogenic bacteria, and this has been attributed to the high volatility of ethanol which tend to extract more active compounds from plant samples than water [29].

The results presented on (Fig. 1) showed that not all the cold aqueous extracts of the two samples had total inhibition on the growth of the tested pathogen at ($p<.05$), this may be due to the inability of the extract to dissolve or diffuse in the media used in the assay Okigbo and Ajale [30] or lack of solubility of active compounds in aqueous solution [31]. Alternatively, active compounds may be present in insufficient quantities in the extracts to show activity with the dose level employed [32].

Although, the hot aqueous extract of *Piper guineense* leaf did not inhibit any of the tested organisms, the hot aqueous extract *Xylopia aethiopica* fruit inhibited *Staphylococcus aureus* but not *Pseudomonas aeruginosa.* Okigbo and Ajale, [30] reported that the inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant material, therefore the lack of inhibitory activity observed with the hot aqueous extracts may thus be concluded to be due to the loss of some active compounds during the extraction process by heat, or there may be lack of solubility of active constituents in hot aqueous solution.

The antibiotics used showed inhibitory effect on the microorganisms tested and the highest zones of inhibition are observed with Ciprofloxacin (Figs. 2-3).

All the spice extracts contained different phytochemicals. It cannot be determined (for now) which of these compounds were responsible for the observed antibacterial activities on (Figs. 1-3). However polyphenols (flavonoids and tannins), which were present in both spices may be responsible for the observed effects because these had earlier been shown to possess bactericidal, fungicidal, virucidal, antiparasitic, insecticidal, medicinal and antioxidant properties [33-36].

The MIC and MBC results of the extracts on "Tables 2 and 3" indicated that the extracts were bactericidal to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This correlates with the work of Nweze and Onyishi [37].

Results of t-test showed that the spice extracts of *Piper guineense* and *Xylopia aethiopica* had significant effect on the microorganisms tested at ($p<.05$).

5. CONCLUSION

In conclusion, the results have shown that extracts of *Piper guineense* and *Xylopia aethiopica* have antibacterial properties and thus can be used as a potential source of natural antimicrobial compounds, as the extracts have lesser side effects which are often associated with the use of antibiotics. Furthermore, the immense challenges associated with antibiotic resistance have made the use of alternative medicine especially, traditional spices an emerging option.

6. RECOMMENDATION

Further work need to be done to determine and identify, purify and quantify the antibacterial compounds within these spices and also to determine their full spectrum of activity as well as their *in vivo* efficacy against disease causing microorganisms before they are used for commercialization in the form of drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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