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Comparison of antimicrobial properties of two spices commonly consumed in Nigeria and effect of temperature on their antioxidant properties

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The study compared the antimicrobial activities of essential oils (EOs) from *Piper guineense* (Uziza) and *Xylopica aethiopica* (Uda) using diffusion and dilution assays, and evaluated their antioxidant properties at different temperatures. Comparison of the antimicrobial activities was determined using well-in-agar diffusion, disc diffusion and broth dilution methods against *Escherichia coli*, *Salmonella* sp and *Bacillus cereus* as test isolates. The antioxidant activities at different temperatures (28, 50 and 70°C) were determined using 1,1- diphenyl-2-picrylhydrazyl (DPPH), ferric ion reducing antioxidant power (FRAP) and Fe²⁺ chelation methods. Their antimicrobial activities increased with increase in concentration. From the diffusion assays, *B. cereus* was more susceptible to the EOs than other isolates (19.5 and 18.0 mm). MIC values obtained from the broth dilution assay (8.0 – 16.0 mg/mL) were generally much lower than those obtained from disc diffusion and well-in-agar diffusion assays. Percent inhibition of isolates using broth dilution method ranged from 91.28 to 96.86%. Their antioxidant activities decreased with increase in temperature. *P. guineense* EO yielded higher DPPH scavenging activity (43.25, 46.64 and 33.36%, respectively) and Fe²⁺ chelating ability (25.60, 12.58 and 8.97 mg/g, respectively) at the different temperatures, while FRAP elicited the strongest antioxidant ability of the EOs (70.10, 37.51 and 26.55 mg/g, respectively). The EOs exhibited their strongest antioxidant abilities at 28°C.

Key words: Microbial isolates, antimicrobial susceptibility testing, antioxidant capacity, spices, essential oils.

INTRODUCTION

Essential oils (EOs) are secondary metabolites which are very rich in compounds with an isoprene structure, called terpenes. They can occur as diterpenes, triterpenes, and tetraterpenes (C₂₀, C₃₀, and C₄₀), or as hemiterpenes (C₅)

and sesquiterpenes (C₁₅). Addition of oxygen to these compounds makes them terpenoids (Cowan, 1999). Several EOs derived from spices elicit varying degrees of antimicrobial effects (Swamy et al., 2016; Cui et al., 2015;

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Sonker et al., 2015; Beatovic et al., 2015) and also possess antioxidant properties (Beatovic et al., 2015) as well as have the ability to suppress cancer cell activity when applied on some human cancer cell lines such as tumors, glioma, breast cancer and leukemia (Adaramoye et al., 2011; Kuete et al., 2015; Sado Kamdem et al., 2015).

The use of essential oils as therapeutic agents and preservatives has become increasingly popular; however, research into the antimicrobial activities of these products has been hampered by lack of standardized and reliable screening methods, especially against food pathogens/food spoilage bacteria. Lack of standardized methods may also make direct comparison of results between studies impossible. Methods for antimicrobial assays are classified into three main groups, which are diffusion (Gulluce et al., 2006), dilution (Passos et al., 2002; Souza et al., 2003) and bio-autographic (Cos et al., 2006; Suleiman et al., 2010) methods. Agar-based disk diffusion is widely used because of its simplicity and low cost. The liquid-dilution method also allows determination of whether a compound or extract has bactericidal or bacteriostatic action at a particular concentration. A simple bioautographic procedure, involves spraying suspensions of the bacteria or fungi on thin layer chromatography (TLC) plates developed in solvents of varying polarities (Suleiman et al., 2010).

Piper guineense (Uziza) (Black pepper) and *Xylopica aethiopica* (Uda) (Guinea pepper) are spices which have over the years become popular amongst Nigerians due to their perceived health benefits. They are used in Nigeria for preparation of different cuisines which are sometimes considered delicacies (Ogueke et al., 2016). The use of these spices in traditional medicinal practice in Nigeria, Africa and other parts of the world are well documented in literature (Nwinyi et al., 2009; Ekanem et al., 2004; N'dri et al., 2009; Ezekwesili et al., 2010; EL-Kamali et al., 2007). In Nigeria and other parts of West Africa, hot extracts of the seeds and leaves of these spices, in addition to other plant parts, serve as tonic for women after childbirth to enhance uterine contraction, prevention of blood coagulation and expulsion of blood from the womb, draining of excess fluid to control weight, as well as an aphrodisiac (Ogueke et al., 2016; Abolaji et al., 2007).

Several researchers have studied the EOs of *P. guineense* from Cameroon and Nigeria (Amvam Zollo et al., 1998; Jirovetz et al., 2002; Tchoumgougnang et al., 2009; Oyediji et al., 2005) which they found to be composed mainly of monoterpenoid alcohol (linalool, 52.2%) (Owolabi et al., 2013). Monoterpenes are the dominant oils present in *X. aethiopica* (El-Kamali and Adam, 2009). Antimicrobial activities of the EOs from these spices have been demonstrated on some microorganisms (Oyediji et al., 2005; Okigbo and Igwe, 2007; Tatsadjieu et al., 2003; Okigbo et al., 2005;

Thomas, 1989; Asekun and Adeniyi, 2004; Schelz

et al., 2006). However, a lot more microorganisms of food and clinical importance require to be screened for susceptibility to the EOs from these spices. The nutritional and health potential of the seed oils of the two spices have been evaluated by some researchers (Ogbonna et al., 2015; Dada et al., 2013) and have been shown to possess low toxicity (Fetse et al., 2016).

There is need to determine the antioxidant activities of these spices with a view to incorporation into food systems especially now that emphasis is on functional foods. It is also important to establish the antioxidant capacity of their EOs at varying temperatures. This is necessary since these spices are used in Nigeria and other African countries for preparation of a variety of dishes that require heating to elevated temperatures (80 to 100°C). Such data will guide users as to the appropriate temperature(s) for incorporation of the spices and their EOs during food processing to maximize their antioxidant capability. These Nigerian spices have been used for centuries as flavouring ingredients in many traditional dishes.

In this study, comparative evaluation of the antimicrobial activities of EOs from *X. aethiopica* and *P. guineense* was conducted using broth dilution assay, disc diffusion and well-in-agar diffusion methods commonly cited in literature for their ability to accurately assess their antimicrobial activities. Their antioxidant properties at varying temperatures were also determined.

MATERIALS AND METHODOLOGY

Collection of spices

Fruits of the Nigerian spices, *X. aethiopica* (Uda) and *P. guineense* (Uziza) were obtained from a farm in Obinze and Ihiagwa, Owerri West Local Government Area (latitude 5° 15'N - 5° 34'N and longitude 6° 52'E - 7° 05'E), Imo State, Nigeria in December, 2016. They were cleaned and sorted to remove sand, spoilt spices and dirt.

Preparation and extraction of essential oils (EOs)

Fruits of *X. aethiopica* and *P. guineense* were destalked, sorted and milled with attrition mill (Landers YCLA, S.A Colombia). The Eos were extracted using the Clevenger hydro distillation method (Selim, 2011). The spices (300 g of each milled spice) were put in a four litre flask (4 L) and 1.5 L distilled water added. The mixture was boiled for 3 h. After boiling, the EOs were collected and dried with anhydrous sodium sulphate. These were then stored at -10°C until required for use.

Preparation of different concentrations of EOs

The EOs were diluted using 5% dimethylsulphoxide (DMSO) solution to obtain the desired concentrations (20 – 100%). These were stirred thoroughly for complete homogeneity. Samples were plated out on nutrient agar and potato dextrose agar plates, and incubated at 37 and 30°C respectively for 24 h and 72 h to ensure sterility of the concentrations prepared.

Test microorganisms

Pure cultures of *Escherichia coli*, *Salmonella* sp. and *Bacillus cereus* were obtained from the laboratories of Federal Medical Centre Owerri (FMC), Imo State, Nigeria. They were used due to their frequent occurrence in clinical samples sent to the laboratories of FMC for analysis. These were re-identified and stored at 4°C on the appropriate agar slants until required for use.

Inoculum standardization

A loopful of the bacterial isolates was inoculated unto Mueller–Hinton Agar (MHA) plates and incubated at 37°C for 24 h. Discrete bacterial colonies were collected using sterile wire loop, inoculated into 10-mL Mueller–Hinton broth and incubated for 30 min. After incubation, the broth was centrifuged for 30 min, the top decanted and broth supernatant was washed with sterilized distilled water. This was centrifuged again and the procedure was repeated three times. After the centrifugation, the microorganisms were harvested and re-suspended in sterilized distilled water in sample bottles. The cultures were adjusted to 0.5 McFarland standards (approx 10^8 cfu/mL) and stored at 4°C, and this was used for the analysis.

Disc diffusion assay

Filter paper No. 1 was perforated to get paper discs of 5 mm diameter. These were sterilized at 110°C for 30 min, impregnated with the concentration of the EOs (20 - 100%) and placed on the surface of the Mueller-Hinton agar plates inoculated with the test bacteria. A control was set up with 0.1 mL dimethylsulphoxide, and the resulting plates were incubated at 37°C for 24 h. The zones of inhibition were measured using a meter rule to the nearest millimeter (mm).

Well-in-agar diffusion assay

This was determined using the method described by Kim et al. (1995) and Lino and Deogracious (2006). 17 mL of Mueller–Hinton Agar (MHA) was poured into sterile petri dishes and allowed to solidify. Using sterile cotton swabs, the cultures of bacteria as prepared above (shown in preparation of microbial strains) were swabbed uniformly on the surface of the plates and allowed to dry for 5 min. All plates were labeled properly and wells of 5.0 mm in diameter were made in the seeded agar plates using a sterile cork borer, sufficiently separated from each other to avoid overlap of growth inhibition zones. 0.01 mL of the different concentrations of the EOs was dispensed into the wells. Three plates were used for each concentration of EO. With the lids on, the plates were allowed to stand for 30 min at ambient temperature (30–32°C) to allow for proper diffusion of the EOs (Ogbulie et al., 2007). The MHA plates were incubated at 37°C for 24 h. The zones of clearance produced around the wells after incubation were observed, measured (diameter of agar well not included) and recorded as the diameter of zone of growth inhibition produced by the EOs. These were carried out in triplicates.

The minimum inhibitory concentrations (MIC) of the EOs using disc diffusion and well-in-agar diffusion assays were determined by plotting a graph of the natural log of concentration of extracts used (X axis) against the squares of the zones of inhibition of the extracts obtained (Y axis). The MIC was read out at the intercept of the regression line of the graph on the X axis. The antilog of the value at the intercept was taken as the MIC (Osadebe and Ukwueze, 2004; Ogbulie et al., 2007).

Broth dilution assay

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of EOs on bacterial isolates

The MIC and MBC of the EOs were determined by the modified method of Salah-Fatnassi et al. (2017), making double fold serial dilutions of the reconstituted extracts (0.625 - 50 mg/mL) in nutrient broth. Bacterial strains with culture suspensions adjusted to 0.5 McFarland standard turbidity (1.5×10^8 cfu/mL) were inoculated into the broth. All culture tubes were incubated at 37°C for 24 h. Growth was scored visually by the turbidity of the culture. The least concentration showing no visible growth was taken to be the MIC.

The MBC was determined by taking 0.1 mL inoculum from the tubes in which there was no growth and sub cultured on Mueller-Hinton agar plates. After incubation at 37°C for 24 h, the plates were observed for bacterial growth. The least concentration showing no growth was taken as the MBC.

Antioxidant properties of EOs

The antioxidant properties of the EOs were determined at 28, 50 and 70°C using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay, ferric ion reducing antioxidant power (FRAP) assay and Fe^{2+} chelation assay.

1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay

Evaluation of the total free radical scavenging capacity of the EOs against DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical was done according to the method described by Gyanfi et al. (1999). The DPPH radical solution was prepared by dissolving 2.4 mg DPPH in 100 ml methanol. A 1:10 dilution of the EOs was prepared and 1 ml of it was mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals. The mixture was shaken vigorously and kept at ambient temperature for 30 min in the dark. Absorbance of the reaction mixture was measured at 516 nm wavelength using a Spectronic Genesys 20 spectrophotometer (Thermo Electron Corporation, USA). DPPH radical without antioxidant was observed and measured. These were performed in duplicate. The ability of the EOs to scavenge the DPPH radical was calculated as stated below using the following equation (Yen and Duh, 1994):

$$\text{DPPH Scavenged (\%)} = ((A_B - A_A) / A_B) \times 100 \quad (1)$$

Where, A_B = absorbance of blank at $t = 0$ min.

A_A = absorbance of the antioxidant at $t = 30$ min.

Ferric ion reducing antioxidant power (FRAP) assay

The ferric reducing activity of the EOs was estimated based on the method (FRAP) developed by Benzie and Strain (1996). The solutions for this assay consisted of the following: 300 mmol/L acetate buffer (pH 3.6), 20 mmol/L ferric chloride and 10 mmol/L TPTZ (2, 4,6-tripyridyl-s-triazine) in 400 mmol/L of HCl. The reagent for the assay was prepared fresh by mixing 10 parts of acetate buffer with 1 part of ferric chloride and 1 part of TPTZ solution. The analysis/assay was performed as follows: 2000 μL of freshly prepared FRAP reagent was mixed with 75 μL of sample, ethanol or hydroalcoholic solvent as appropriate for reagent blank. The absorbance was read at 593 nm using Spectronic Genesys 20 spectrophotometer (Thermo Electron Corporation, USA) after 30 min of incubation. The values obtained were expressed as ascorbic acid equivalent.

Table 1. MIC of *X. aethiopica* and *P. guineense* EOs on isolates using Disc diffusion and Well-in-Agar diffusion methods.

Essential oils	Microorganism	MIC (mg/mL)	
		Disc	Well-in-Agar
<i>P. guineense</i>	<i>B. cereus</i>	234.42	1.00
<i>P. guineense</i>	<i>E. coli</i>	81.28	69.18
<i>P. guineense</i>	<i>Salmonella</i> sp	128.82	251.19
<i>X. aethiopica</i>	<i>E. coli</i>	251.19	69.18
<i>X. aethiopica</i>	<i>Salmonella</i> sp	173.78	141.25
<i>X. aethiopica</i>	<i>B. cereus</i>	3.39	1.20

Fe²⁺ chelation assay

The Fe²⁺ chelating ability of the EOs was determined using the method of Minotti and Aust (1997), however, with slight modification by Puntel et al. (2005). Freshly prepared 500 µM FeSO₄ (150 µL) was added to a reaction mixture containing 168 µl 0.1 M Tris- HCl (pH 7.4), 218 µL saline and 100 µL of a 1:10 dilution of the essential oils. This was incubated for 5 min, before the addition of 13 µL 0.25% 1, 10- phenanthroline solution (w/v). The absorbance was subsequently measured at 510 nm wavelength; thereafter, the Fe²⁺ chelating ability was calculated.

Statistical analysis

The data were means of triplicate determinations and were analysed using Analysis of Variance (ANOVA). Statistical Package for Social Scientists (SPSS) version 20.0 was used for statistical analysis of data obtained. The means were separated using Fisher's Least Significant Difference (LSD) at 95% confidence level (p<0.05).

RESULTS

Antibacterial activity of EOs against isolates

Antibacterial susceptibility testing of *X. aethiopica* and *P. guineense* EOs against test isolates using well-in-agar diffusion and disc diffusion methods are shown in Figures 1 and 2 respectively, and Table 1. With well-in-agar diffusion method, *B. cereus* was observed to be more susceptible to the activity of both spices with a zone of growth inhibition measuring 19.5 and 18.0 mm for *P. guineense* and *X. aethiopica* EOs respectively at 100% concentration (Figure 1). However, the EOs had lower activities on *E. coli* both having a zone of growth inhibition of 9 mm at 100% concentration. *E. coli* was resistant to the activities of the EOs at concentrations lower than or equal to 33.3% of the EOs. Similar trend in antimicrobial activities was recorded using the disc diffusion method (Figure 2) although the values obtained were lower than those obtained with the well-in-agar diffusion method.

MIC values of the EOs obtained from disc diffusion and well-in-agar diffusion assays are shown in Table 1. The

MIC values were generally high. The highest value (251.19 mg/mL) was produced by *P. guineense* and *X. aethiopica* on *Salmonella* sp. and *E. coli* respectively. With well-in-agar diffusion assay, *P. guineense* and *X. aethiopica* produced the lowest MIC values of 1.00 and 1.20 mg/ml respectively on *B. cereus*. However, with the disc diffusion assay, the EOs produced MIC values of 234.42 and 3.39 mg/mL respectively on the same bacterium.

Broth dilution assay

Results from the broth dilution assay showed MIC value of 8.0 mg/mL on the test isolates except *E. coli* where the activity of *P. guineense* produced MIC of 16.0 mg/mL. The MBC values ranged from 16-32 mg/mL (Table 2). Such low MIC values are of significance. Figures 3 to 8 show the graphs of percentage inhibition against concentration of EO. These revealed that the percentage inhibition increased with increase in concentration of the EOs. The values ranged from 91.28% as observed on *B. cereus* produced by *P. guineense* to 96.86% on *Salmonella* sp. produced by *X. aethiopica* at concentration of 32 mg/mL. For *E. coli*, the maximum percent inhibition was achieved at 94% at concentration of 32 mg/mL by *X. aethiopica* EO and 96% at a concentration of 32 mg/mL by *P. guineense* EO respectively (Figures 3 and 4). This further justifies the results obtained from the MIC and MBC using broth dilution assay.

Antioxidant properties of the EOs

The antioxidant properties of the EOs at different temperatures (28, 50 and 70°C) using the three methods (Tables 3 and 4) show that their activities decreased with increase in temperature. The abilities of the EOs to scavenge DPPH at different temperatures for *P. guineense* EO were 43.25, 28.06 and 23.89% respectively while *X. aethiopica* EO were 38.02, 25.09 and 21.55% respectively. *P. guineense* EO yielded higher DPPH

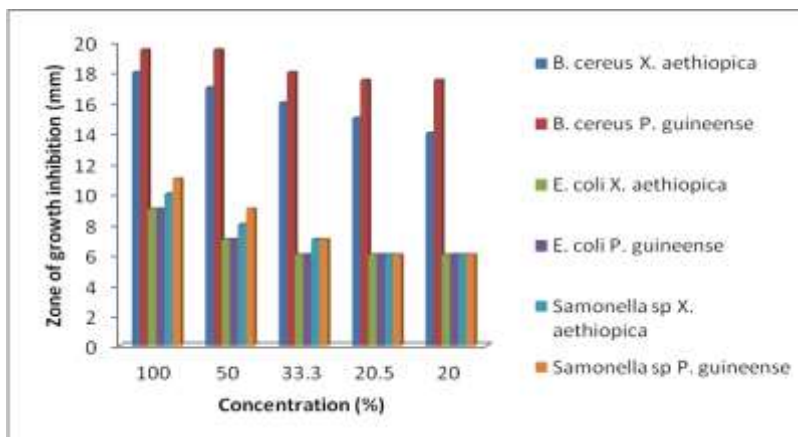


Figure 1. Antimicrobial susceptibility testing using well-in-agar diffusion method.

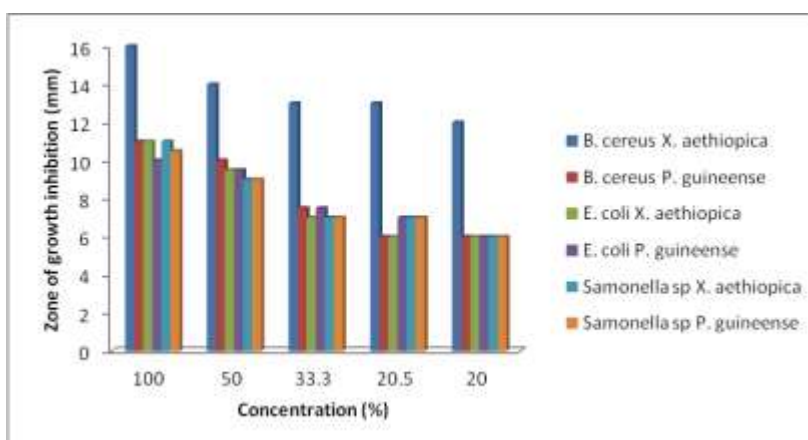


Figure 2. Antimicrobial susceptibility testing using disc diffusion method.

scavenging activity at the different temperatures than *X. aethiopica* EO. Fe^{2+} chelating ability of *P. guineense* EO was also higher at the temperatures studied (25.60, 12.58 and 8.97 mg/g, respectively) than the *X. aethiopica* EO. However, for the FRAP, *X. aethiopica* EO yielded higher reducing property than *P. guineense* EO with values of 70.10, 37.51 and 26.55 mg/g at 28, 50 and 70°C, respectively.

The values obtained significantly differed ($p < 0.05$) among the methods and the temperatures adopted for the study. FRAP elicited the strongest antioxidant ability of the EOs while the optimum temperature at which the EOs exhibited the strongest antioxidant ability was 28°C.

DISCUSSION

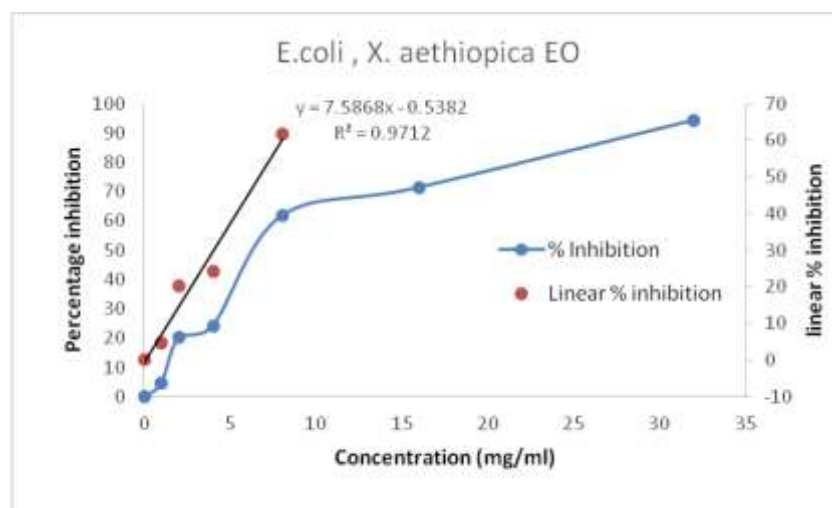
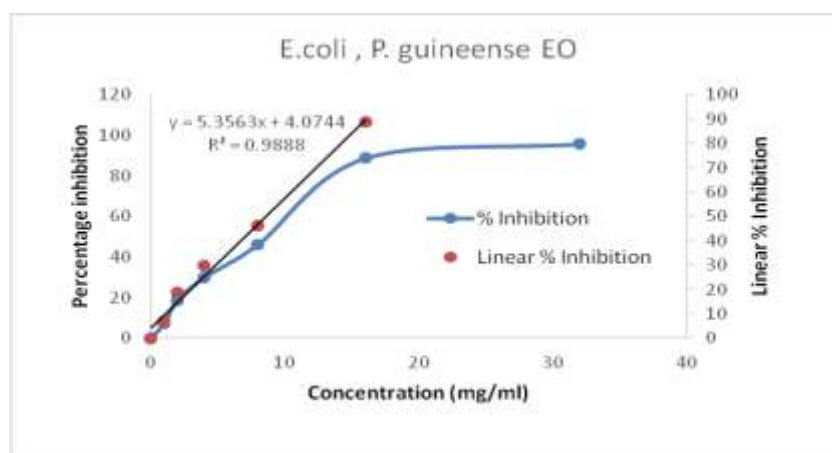
Antimicrobial activities of extracts of spices have been studied by different researchers and their activities have been attributed to the phytochemicals they contain. The

EOs tested in this study showed antimicrobial activity (bactericidal and bacteriostatic) on all the tested isolates which are common food pathogens (*E. coli*, *Salmonella* sp. and *B. cereus*), although differences were observed between their antimicrobial activities (Figures 1 and 2, Tables 1 and 2). Composition of critical chemical compounds in spices differs significantly due to variations and differences in genetic makeup, agronomical practices and environmental factors; thus, they may bring about differences in their antibacterial efficacy against food borne pathogens (Cowan, 1999; Chao et al., 1999; Hao et al., 1998). Such EOs could therefore be applied as antimicrobial preservatives in food (Pandey et al., 2017), although information in this line is minimal.

This study has shown that broth dilution assay (Table 2) is a better protocol for determining the antimicrobial efficacy of EOs from these spices than disc diffusion or well-in-agar diffusion methods (Table 1). MIC values obtained from the broth dilution assay were generally much lower than those obtained from disc diffusion and

Table 2. MIC and MBC of *X. aethiopica* and *P. guineense* EOs on isolates.

Essential oils	Microorganism	MIC (mg/mL)	MBC (mg/mL)
<i>P. guineense</i>	<i>B. cereus</i>	8	16
<i>P. guineense</i>	<i>E. coli</i>	16	32
<i>P. guineense</i>	<i>Salmonella</i> sp	8	32
<i>X. aethiopica</i>	<i>E. coli</i>	8	32
<i>X. aethiopica</i>	<i>Salmonella</i> sp	8	16
<i>X. aethiopica</i>	<i>B. cereus</i>	8	32

**Figure 3.** Graph of percentage inhibition against concentration of *X. aethiopica* on *E. coli*.**Figure 4.** Graph of percentage inhibition against concentration of *P. guineense* on *E. coli*.

well-in-agar assays, thus indicating a stronger antibacterial activity against the tested isolates. The rate of diffusion in agar media and thickness of oils play a

major role in the use of these protocols in assessing antimicrobial activities of oil containing extracts of plants such as the EOs from *P. guineense* and *X. aethiopica*.

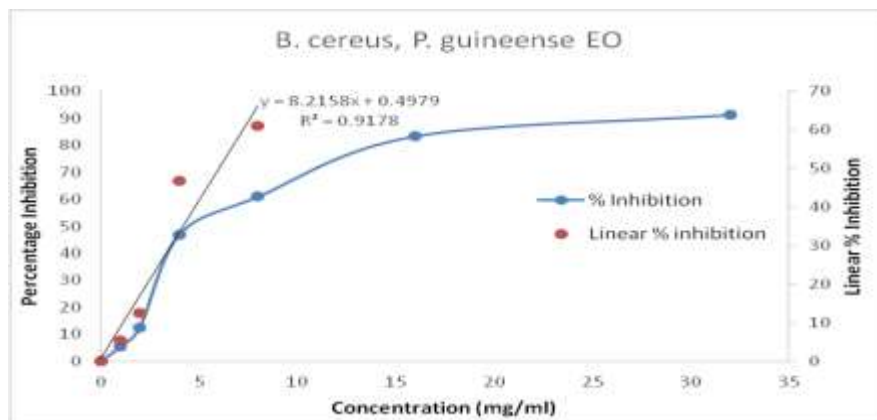


Figure 5. Graph of percentage inhibition against concentration of *P. guineense* on *B. cereus*.

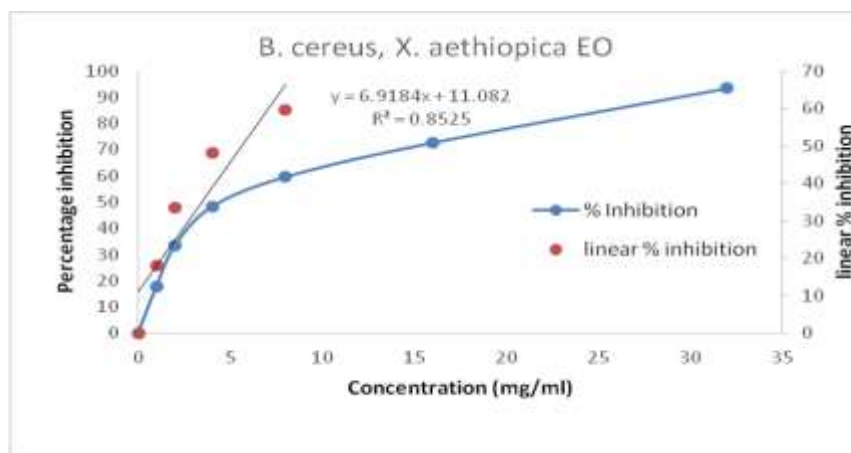


Figure 6. Graph of percentage inhibition against concentration of *X. aethiopica* on *B. cereus*.

Insolubility of active compounds in water and culture medium or the presence of inhibitors to the antimicrobial components also plays major roles in the activity of such EOs (Burt, 2004; Janssen et al., 1987; Kalemba and Kunicka, 2003; Okigbo and Ogbonnanya, 2006; Scorzon et al., 2007). EOs are complex mixtures of a wide variety of components. Thus, their antimicrobial activity is related to their configuration, composition, amount and possible interaction which could be additive, antagonist or synergetic (Lis-Balchin et al., 1998; Wang et al., 2016). Inactivity of the EOs and other extracts of plants may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials (Okigbo et al., 2005; Okigbo and Ajalie, 2005). This corroborates the findings of Hadacek and Greger (2000) who reported that serial dilution test gave the most reproducible results for MIC and thus was recommended as a general standard methodology for testing of natural products.

Phytochemicals found in extracts of plants such as phenolics had been known to exhibit strong radical scavenging activity. Phenolic compounds in some cases have been found to be synergistic in their actions where they prevent the formation of reactive O_2 species, hydroxyl radicals, superoxide anions and hydrogen peroxide which usually cause oxidative damage to biomolecules (Satish et al., 2014). The study revealed that the EOs exhibited potent antioxidant properties (Tables 3 and 4). However, using the FRAP method, *X. aethiopica* EO exhibited the strongest reducing power. Such plant materials with high reducing power are usually good source of natural antioxidants. The antioxidant properties recorded for the EOs could be attributed to the bioactive compounds, phytochemicals and pigments they possess. *P. guineense* has been reported to contain saffrole, 5-8% piperine, elemicine, 10% myristicine and dillapiol (Osuala and Anyadoh, 2006).

The observation that the antioxidant capacities of the

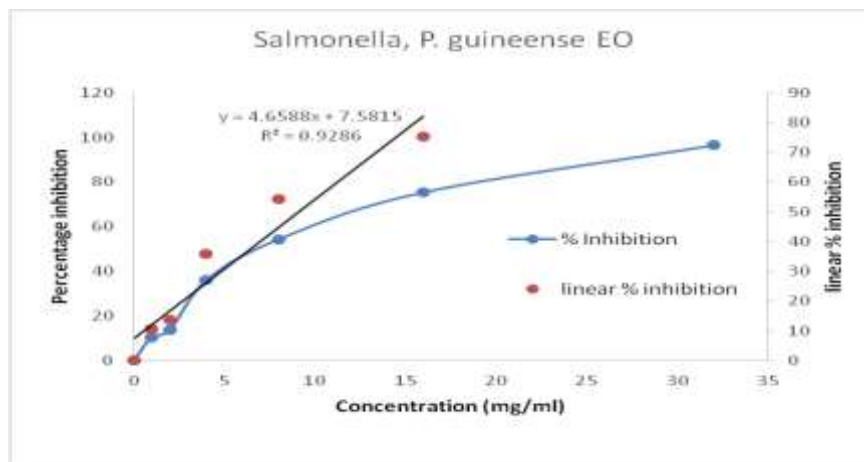


Figure 7. Graph of percentage inhibition against concentration of *P. guineense* on *Salmonella* sp.

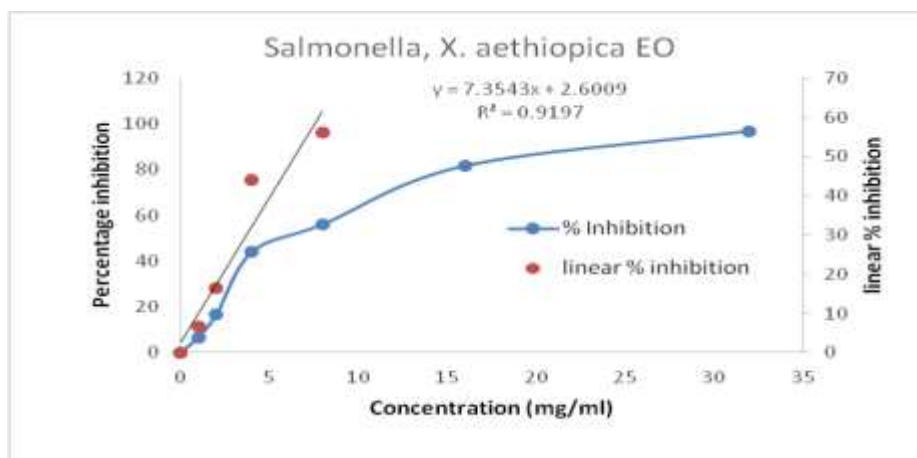


Figure 8. Graph of percentage inhibition against concentration of *X. aethiopica* on *Salmonella* sp.

EOs decreased with increase in temperature implies that when included in foods as preservatives such foods should be stored at temperatures not exceeding 28°C so as to obtain their maximum capabilities as antioxidants. Weaker antioxidant activity at higher temperatures could be attributed to the volatile characteristics of components of the EOs which are simultaneously lost through evaporation as temperatures increased. Polyphenolic compounds are known to boost antioxidant properties of natural extracts (Sun et al., 2007), thus increase in temperature could lead to loss of these polyphenolic compounds with attendant loss of antioxidant activity. The implication of this loss of antioxidant activities at higher temperatures is that food preparations requiring high temperature processing as is common with most dishes prepared with these spices in Nigeria, and other African countries may not provide the needed free radical

scavenging/antioxidant ability. It is necessary, therefore, to develop method(s) for addition of the spices or their EOs after heating of the food product to ensure the antioxidant ability is not lost.

Conclusion

This study has demonstrated that the EOs from these spices (*X. aethiopica* and *P. guineense*) possess varying degrees of antimicrobial activities and antioxidant properties. Comparison of antimicrobial activities using well-in-agar diffusion, disk diffusion and broth dilution methods showed that broth dilution method/assay was a better protocol for determining the antimicrobial efficacy of the EOs from these spices than disc diffusion or well-in-agar methods. Analysis using different methods

Table 3. Antioxidant property of *P. guineense* EO at different temperatures (°C).

Method	Temperature (°C)			LSD
	28	50	70	
Fe ²⁺ (mg/g)	¹ 25.60 ^c ±0.08	² 12.58 ^c ±0.01	³ 8.97 ^c ±0.01	0.144
DPPH (%)	¹ 43.25 ^b ±0.01	² 28.06 ^b ±0.01	³ 23.89 ^b ±0.01	0.023
FRAP (mg/g)	¹ 48.51 ^a ±0.01	² 46.64 ^a ±0.08	³ 33.36 ^a ±0.01	0.146
LSD	0.032	0.144	0.023	

^{abc} Values with different superscripts along the columns differ significantly (p< 0.05)

¹²³⁴ Values with different superscripts on the same row are significantly different (p< 0.05)

KEY:

DPPD = 1,1- diphenyl- 2- picrylhydrazyl free radical scavenging ability.

FRPA = Ferric ion reducing power assay.

Fe²⁺ = Ferrous chelating ability.

Table 4. Antioxidant property of *X. aethiopica* EO at different temperature (°C).

Method	Temperature (°C)			LSD
	28	50	70	
Fe ²⁺ (mg/g)	¹ 20.10 ^c ±0.01	² 10.26 ^c ±0.01	³ 7.55 ^c ±0.01	0.131
DPPH (%)	¹ 38.02 ^b ±0.01	² 25.09 ^b ±0.02	³ 21.55 ^b ±0.01	0.136
FRAP (mg/g)	¹ 70.10 ^a ±0.01	² 37.51 ^a ±0.01	³ 26.55 ^a ±0.01	0.023
LSD	0.023	0.043	0.225	

^{abc} Values with different superscripts along the columns differ significantly (p< 0.05)

¹²³⁴ Values with different superscripts on the same row are significantly different (p< 0.05)

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DPPD = 1,1- diphenyl- 2- picrylhydrazyl free radical scavenging ability.

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Fe²⁺ = Ferrous chelating ability.

showed that FRAP elicited the strongest antioxidant ability of the EOs. The antioxidant properties were also temperature dependent and exhibited their strongest antioxidant ability at 28°C. Further studies aimed at application of the EOs in food matrix are recommended to establish their actual activities in a food system.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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