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Microbial and Antioxidant Activities of Some Common Spices from Southeast Nigeria

P. C. Ojimelukwe[1*] and A. N. Ukom[1]

[1]Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author PCO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author ANU managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The aim of this research was to evaluate the antioxidant and antimicrobial properties of *Piper guineense, Xylopia aethiopica, Tetrapleura tetraptera, Monodora myristica* Njameja. The total phenol contents and antioxidant activities of the indigenous spice extracts were determined using Folin-Ciocalteau´s reagent; ABTS (2,2´-azinobis-(3-ethylbenzothiazoline-6-sulponic acid) diammonium salt; DPPH (2,2-diphenyl-1-picrylhydrazyl) and ORAC (Oxygen Radical Absorbance Capacity). Phenolic content of the spices was correlated with the anti-oxidant properties. Antimicrobial properties of some of the spices against known food spoilage and pathogenic microorganisms were also determined. *Xylopia aethiopica*, *Tetrapleura tetraptera, Piper guineense*, *Monodora myristica*, and Njameja contained high levels of total phenols and strong antioxidant activity. Total phenolic content ranged from 869.11 to 1536.40 mg GAE/100g/dw. Antioxidant activity measured by ABTS varied from 1043.74 to 5978.50 mg Trolox Equivalent (TE)/100g/dw, DPPH from 1165 to 13038.92 mg Trolox Equivalent (TE)/100g/dw, and ORAC, from 261.05 to 379.28 ± 2.14 mg Trolox Equivalent (TE)/100g/dw respectively. A positive linear correlation was obtained between total phenol content and ABTS (R^2= 0.854). Correlation coefficient between total phenolic content and DPPH was lower (R^2= 693), and even lower between total phenolic content and ORAC (R^2= 549). The assay methods correlated well with each other (R^2= 828-932). The positive linear correlation between total phenolic content and antioxidant activity values show that

**Corresponding author: E-mail: philippaco60@gmail.com;*

phenolic compounds in the tested spice extracts contributed significantly to the strong antioxidant activity. In this study, *Xylopia aethiopica* and *Tetrapleura tetraptera* showed higher values for phenolic compounds and higher antioxidant activities indicating that they can scavenge free radical actions and prevent lipid peroxidation. *X. aethiopica* showed bactericidal properties against *S. aureus, Salmonella*, *Klebsiella*, *Lactobacillus* and *E. coli*. None of the spices was inhibitory to fungi at concentrations used for the experiments. Further analysis is required to decipher the nature of their phenolic constituents with a view to determining the potentials of these natural antioxidants for commercial ssexploitation.

Keywords: Spice extracts; total phenolic content; antioxidant activity; free radical actions.

1. INTRODUCTION

Spices are vegetables**,** seeds and fruits that are used as food adjuncts to season, flavor, and preserve foods throughout the world. Many spice plants are also medicinal herbs that are used in traditional medicine. They spread across Asia, America, Europe and Africa. Many common spices are involved in trans-border trades. They include among others, rosemary (*Rosmarinus officinalis L.),* ginger *(Zingiber)*,parsley *(Petroselinum crispum L.),* dill *(Anthum graveolens L.),* chilli *(Capsicum annuum L.),* nutmeg *(Myristica fragrans Houtt.),* thyme etc*.* [1]. In Africa, especially in the rain forest zone of Nigeria, Ghana and Cameroun, some indigenous spices and herbs have found much use in food preparations. They are *Xylopia aethiopica* (African pepper), *Monodora myristica* (African nutmeg/Calabash nutmeg), *Piper guineense* (West African pepper), *Tetrapluera tetraptera* (Oshosho, Igbo), *Ocimum gratissimum* (Scent leaf) etc. These spices are used to prepare sauce, soup and porridge during the cold season due to their hot and peppery taste [2]. Hot aqueous extracts of *Tetrapleura tetrptera, Xylopia aethiopica* and *Piper guinneense* are prepared as restorative soup and sometimes with yam (*Dioscorea rotundata)* for post-partum women to aid contraction of the uterus [3]. It is believed by the people of South Eastern States of Nigeria that these spice extracts or their restorative soup serve some important roles for lactation, afterbirth cleansing of the women´s womb and restoration of the women´s tummy to normal shape after childbirth within a period of three months.

Generally, significant quantities of phenolic compounds occur in foods such as fruits, vegetables, and seeds. It is reported that phenolic compounds of fruits and vegetables have antioxidative, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and anti -inflammatory activities [4,5]. Phenolic compounds in diets have good scavenging activity against peroxy and superoxide anion radicals [6,7]. Some phenolic compounds in spice extracts (monoterpene hydrocarbon, flavonoids, phenolic acids and phenolic amides, volatile oils and coumarins) have been identified to possess strong antioxidant capacities against free radical actions and render several health benefits [1,7,8]. Also, spices and medicinal herbs are used in folk medicine for the treatment of hypertensive disorders, inflammation and several women´s diseases such as breast and uterus cancer [9], and in diabetic and cardiovascular treatments [10]. Spice extract s have potentials for strong digestive stimulation, antimicrobial action and lipid peroxidation necessary to extend the shelf life of foods [11]. Spice antioxidants could be used as potential alternatives to other sources of antioxidants to improve human health. Therefore antioxidant compounds in spices may play important roles in the synergistic contribution to total antioxidant activity. The evaluation of the total phenolic content and antioxidant activity of some commonly used spice extracts will lead to the understanding of their potential health benefits. It has been established that antioxidant compounds from fruits and vegetables are more healthful than the synthetic antioxidants that can promote tumor apoptosis [12,13].

The present study aims at evaluating the phenolic content, antioxidant activity and antimicrobial properties of some spices commonly used in food preparations in Nigeria, to establish the relationship between antioxidant activity and phenolic content of the spice extracts.

2. MATERIALS AND METHODOLOGY

2.1 Materials

The most commonly used parts (seed, leaf and fruit) of six common spices were used for this

analysis. Their family, scientific names, common names and parts used are listed in Table 1.

2.2 Sample preparation

The dried spices were obtained from Ubani main Market in Umuahia North LGA, Abia State, Nigeria. The spice samples were cleansed to remove straw, foreign bodies and sorted to remove unwanted ones. They were pulverized using Thomas Wiley Laboratory Mill (Model 4, USA), and sieved using 40 (0.42 mm) mesh size. The powdered spice samples were packaged in separate cellophane bags and stored at 4℃ for subsequent use.

2.3 Extraction of Phenolic compounds

Acetone/water (50:50, v/v) was the solvent used for extraction.

About 0.5 gram of powdered spice samples (triplicate) was mixed with 5 mL of the extraction solvent in a 50 mL BD Falcon tubes using Ultra Turax (1Ka T18 basic Staufen, Germany) for 10 seconds and then capped and re-mixed in a Vortex mixer (Fisher Scientific, USA) for 1 minute. The spice samples were placed in a multi-purpose rotator (Barnstead International, USA) for 30 minute; then centrifuged at 4℃ for 5 minutes at 6000 rpm, (Eppendorf Centrifuge 5804R Hamburg, Germany). The spice extracts (2 mL), each was collected and stored in the dark at 4℃ for determination of total polyphenol, ABTS, DPPH and ORAC.

2.4 Determination of Total Phenols

Total Polyphenols (TP) content was determined using the Folin-Ciocalteu method according to Jayaprakashan et al. [14] with minor modification. 780 µl distilled water, 20 µl spice extract and 50µl Folin-Ciocalteau reagent (1:1 with water) were added and mixed in a 2 mL Eppendorf tube. After 1 minute, 150 µl sodium carbonate (Na2Co3) (0.2 g/mL) was added, and the mixture was allowed to stand at room temperature (25℃) in the dark for 1 h. Then, 300 µl of the mixture was carefully introduced into a 96 microtiter well plate. The absorbance was read at 750 nm (µQuant, Biotech Instruments, USA). The total polyphenols concentration was calculated from a calibration curve, using Gallic acid (1 mg/ml) as standard (200 –1000 mg/L).

2.5 Measurement of Antioxidant Capacity Antioxidant Capacity Determined by DPPH

The DPPH free radical scavenging activity was determined according to the method of Brand-Williams et al. [15] with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and then stored at – 20℃ over-night. The working solution was obtained by mixing 10 mL stock solution with 45 ml methanol to obtain an absorbance of 1.1 - ±0.0.2 units at 517 nm using the spectrophotometer (µQuant, Biotech Instruments, USA). Spicesample extracts (100 µL) were allowed to react with 1900 µL of the DPPH solution for 1h. Then 300 µL of the reaction mixture was added into 96 microtiter plate and read at 517 nm. The standard curve was linear between 150 – 500 µM Trolox (0.5 mM concentration). Results were expressed in mgTE/g fresh weight. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

2.6 Antioxidant Capacity Determined by $ABTS^{\cdot+}$ Radical Cation

For ABTS assay, the procedure followed the method of Arnao et al. [16] with some modifications. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting 38.4 mg ABTS and 6.6 mg potassium persulphate in 10 mL of distilled water and allowing the mixture to stand in the dark at room temperature (25℃) for 16h before use. The $ABTS^{\cdot+}$ Stock solution was diluted with ethanol to obtain an absorbance of 1.1 ±0.02 at 734 nm. 30 µL of spice sample extract or Trolox standard and 200 µL $ABTS^{\cdot+}$ solution were added together and allowed for 6 minutes before reading at 734 nm (µQuant, Biotech Instruments, USA). Results were expressed as Trolox equivalent fresh weight (TE/gFW). The Trolox standard curve was linear between 50 – 400µM Trolox.

2.7 Antioxidant Capacity Determined by Oxygen Radical Absorbance Capacity (ORAC)

The ORAC assay was performed as described by Huang et al. [17]. 130mg AAPH was dissolved in 3 ml PBS of 75mM (pH7.4) to a final concentration of 153mM and made fresh daily. A fluorescein stock solution (4x10-3 mM) was dissolved in 75 mM PBS (pH 7.4) and stored at

4℃. The fluorescein stock solution was diluted 1:1000 with PBS (pH7.4). To the experimental 96 wells, 150 µL fluorescein diluted solution was added. In addition, blank wells received 25 µL of 75 mM PBS (pH 7.4), while standards received 25 µL of Trolox dilution and sample wells received 25 µL of sample extracts (diluted at 1:100). Reaction was initiated by adding 25 µL of AAPH reagent with a shake duration of 8 seconds. A Multi-Mode Microplate Reader, Instrument INC, USA, with injectors was used with 485/20 nm excitation filter and a 530/25 nm emission filter. The number of Kinetic cycles was 30 and Kinetic interval was 60 seconds.

The ORAC values were calculated as described by Cao and Prior [18]. The Area under the Curve (AUC) and the Net AUC of the standards and samples were determined using Equations (1) and (2), respectively. Results were expressed as trolox equivalent fresh weight (TE/gFW)

$$AUC = 0.5 + (R_2/R_1) + (R_3/R_1) + (R_4/R_1) \ldots\ldots + 0.5\ (R_n/R_1) \quad (1)$$

Where R1 is the fluorescence reading at initiation of the reaction and Rn is the lastmeasurement.

$$\text{Net AUC} = \text{AUC sample} - \text{AUC blank} \quad (2)$$

2.8 Determination of Antimicrobial Activities against Selected Pathogens

The microorganisms used for this study were obtained in pure cultures by continuous culturing and sub-culturing from fermenting legumes (Oil bean, melon and castor bean seeds). Mackonkey agar, Sabouraud dextrose agar and potato dextrose agar were used for fungal isolation as appropriate. Nutrient agar was used for the isolation of bacteria. Incubation was carried out at 27 C and 5 days for fungi and at 37 C and 2 days for bacteria. Biochemical tests were used to identify the microorganisms (catalase test, oxidase test, sugar fermentation tests, indole test, gram stain reaction, lactophenol test and spore test) [19]. *E. coli*, *Staphylococcus*, *B. subtilis*, *Lactobacillus*, and *Klebsiella* were the bacteria used while *Rhizopus* and *Aspergillus* species were the fungi used. Inhibition tests were carried out by inoculating bacteria into fresh nutrient agar infused with spice powders (0-1 g) at 37℃. They were examined for inhibition for up to 72h. . Fungicidal activity was determined by incubating the isolated test organism with the media containing potential inhibitors (i.e. spice extracts at 27℃ and examining the culture for inhibition for up to 72 h. Cultures were visually observed for zones of inhibition.

2.9 Statistical Analysis

Data analysis for this paper was generated using SAS software. (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was done using PROD. GLM in SAS (SAS, 2003). Means were separated using LSD at $P<0.05$. Pearson Correlation analysis was carried out using SAS (SAS, 2003) [20].

3. RESULTS AND DISCUSSION

3.1 Total Phenols

Total phenol content of the spice extracts varied widely, ranging from 353.86 to 1536.39 mgGAE/100 gdw, with a mean of 1033.5 mgGAE/100 g/dw. *Xylopia aethiopica* (1536.39 mgGAE/100 gdw) and *Terapluera teraptera* (1424.0 mgGAE/100g dw) exhibited the highest phenolic content, and were more than four times the value of *Monodora myristica* (353.86mgGAE/100gdw), which had the lowest value. *Piper guineense* (both leaf and seed) (1038.7 and 978.86 mgGAE/100 gdw) and *Njameja* leaf (869.1 mgGAE/100 g dw) also contained high levels of phenols. The results indicated that *Xylopia aethiopica* and *Terapluera teraptera* which exhibited the highest phenolic content also showed strongest antioxidant activities (Table 2). Comparing with reports of total phenolic content and total antioxidant activity (TEAC) of 26 common spices [1], and antioxidant activity of 30 spice extracts that are used frequently in ready meals [8], this study indicated higher phenolic content data also showed that total phenolic content and antioxidant activity from the spice extracts are stronger than the reported values of plum cultivars [5], guava fruit extracts [21], *Actinidia* fruits [22]. In agreement with our findings, Du et al. [22] reported that phenolic compounds are responsible for the antioxidant activity of fruits and vegetables.

3.2 Antioxidant Properties of Spice Extracts Measured Using Different Assay Methods

The antioxidant activity of the spice extracts varied significantly ($p<0.05$). *Xylopia aethiopica* and *Terapluera teraptera* exhibited the strongest

antioxidant activities measured by ABTS, DPPH and ORAC assays.

3.2.1 ABTS antioxidant activity

The free radical scavenging activity determined by ABTS ranged from 1034.74 to 5978.5 mgTrolox Equivalent (TE)/100 g DW, with a mean value of 2800.12 mgTE/100 g DW. *Xylopia aethiopica* (5978.5 mgTE/100 g DW) and *Terapluera teraptera* (5457.51 mgTE/100 g DW) possessed the strongest antioxidant activity and were more than three times higher than the value of *Piper guineense* (leaf and seed), and more than five times higher than the value of *Monodora myristica* and *Njameja* leaf.

3.2.2 DPPH antioxidant activity

The antioxidant activity of the spice extracts assayed by DPPH showed strong scavenging potentials (Table 2). There were significant differences ($p<0.5$) in the values obtained. The scavenging potential varied from 1165.56 to 13038.92 mgTE/100 g DW, with a mean value of 6692.1 mgTE/100 g DW. In this assay method, *Terapluera teraptera* was stronger than other spice extracts. This was followed by *Xylopia aethiopica* (12760.58 mgTE/100 g WD), *Piper guineense* seed (6011.86 mgTE/100 g DW) and *Monodora myristica* (4777.35 mgTE/100 g DW), while *Njameja* leaf (2398.37 mgTE/100g DW) and *Piper guineense* leaf (1165.56 mg TE/100 g DW) were the weakest. The result indicates that *Terapluera teraptera* and *Xylopia aethiopica* were eleven times stronger than *Piper guineense* and five times stronger than the antioxidant activity of *Njameja*leaf. The observation was that spice seeds and fruit extracts demonstrated higher DPPH scavenging power than the leaf extracts probably due to the contribution of volatile oils to antioxidant activity that is present in the seed extracts.

3.2.3 ORAC antioxidant activity

The antioxidant activity measured by ORAC-Fluorescein method is shown in Table 2. The ORAC values of the spice extracts ranged from 261.0 to 379.28 mg TE/100 g DW, with a mean value of 313.94 mgTE/100 g DW. The ORAC value of *Xylopia aethiopica* (379.28 mgTE/100 g DW) was significantly ($P<.0.05$) higher than other spice extracts, followed by *Terapluera teraptera* (349.0 mgTE/100 g DW), *Piper guineense* seed (316.74 mgTE/100 g DW) and *Monodora myristica* (311.42 mgTE/100 g DW). The spice seed extracts show higher ORAC scavenging activity than the leaf spice extracts of *Njameja* leaf (266.12 mgTE/100 gDW) and *Piper guineense* leaf (261 mgTE/100 gDW). ORAC-Fluorescein assay is sensitive, and can measure the combined hydrophilic antioxidants (polyphenol) and lipophilic antioxidants (volatile oils and α-tocopherol) in the spice seed and fruit extracts.

There is an inverse relationship between phenolic compounds and the risk of oxidative stress and lipid oxidation *in vitro* [5,23]. The high content of phenolic compounds and very strong antioxidant activity of the spice extracts indicates its strong scavenging power against free radical actions. There is an indication that the phenolic compounds in the spice extracts specifically phenolic acids and flavonoids which are contained in spices [1] may possess high numbers of hydroxyl groups or hydrogen donating groups in their aromatic ring structure that could lead to stronger antioxidant activity. Antioxidant compounds acts in different ways in the oxidative sequence of lipid molecules. They can intercept singlet oxygen and prevent initiation reaction of oxidative degradation of lipids because of their ability to act against a wide range of free radical cations in multiple hydroxyl groups [24-26].

Culturally, women in Southeastern Nigeria strongly believe in the use of the extracts and/or soup/sauce of *Xylopia aethiopica, Terapluera teraptera* and *Piper guineense* as a remedial medicine for after birth cleansing of the women´s womb. During pregnancy, there is increase in body fat accumulation associated with both hyperphagia and increased lipogenesis which are mediated through hormonal changes and dietary practices. Also during lactation, the woman's body changes in response to neuroendocrine and biochemical stimuli that may be affected by nutritional and environmental factors and may increase adiposity [27,28]. Our study show that spice extracts of *Xylopia aethiopica, Terapleura teraptera* and *Piper guineense* from this region have high antioxidant content and strong scavenging power, and possibly strong lipid peroxidation. This may provide preliminary information on the application of these spice extracts as after birth cleansing of the women´s womb and to restore the body weight changes to normal few months after birth. Although this claim is subject to further verification by *in vivo* trials, it works for the local women as an age long practice in South east Nigeria probably due to the spices strong

antioxidant and lipid peroxidation activities, that may break down adipose fat deposit without side effects.

3.3 Correlation of Phenolic Content of Compounds and Antioxidant Activity

In order to show the contribution of phenolic compounds to antioxidant activity, correlation analysis was performed on the spice extracts. ABTS, DPPH and ORAC were highly correlated with total phenols in this study (Table 3). A positive linear relationship was obtained by total phenolic content and ABTS (R^2=0.854), between total phenolic and DPPH (R^2=0.693), and between total phenolic and ORAC (R^2=0.549). On the assay methods, there was high correlation between ABTS and DPPH (R^2=0.932), ABTS and ORAC (R^2=0.828). The total phenolic content and antioxidant activity values showed that phenolic compounds in the tested spice extracts contributed significantly ($p<0.05$) to antioxidant activity. In this study, the total phenolic content and antioxidant activity of *Xylopia aethiopica* (Uda) and *Terapluera teraptera* (Oshosho) showed higher values and could pose as potential sources of potent natural antioxidants. The indication is that phenolic compounds in this selected spice extracts can scavenge free radicals and prevent lipid peroxidation.

3.4 Identification of Bacteria Used for Inhibition Studies

The probable identity of bacteria used for inhibition studies are shown in Table 4. *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, *Lactobacillus and Klebsiella*, were identified and used. These bacteria are pathogenic or spoilage microorganisms found in foods.

Table 1. Spices used for experiments

S. no/Family	Scientific name	Common name	Part used
1. Anonaceae	*Xylopia aethiopica*	African pepper, Uda (Igbo)	Seed
2. Anonaceae	*Monodora myristica*	African nutmeg, Calabash nutmeg, Ehuru (Igbo)	Seed
3. Piperaceae	*Piper guineense*	West African pepper, Uziza (Igbo)	Seed
4. Piperaceae	*Piper guineense*	West African pepper	Leaf
5. Leguminoseae	*Tetrapleura tetraptera*	Oshosho (Igbo)	Fruit
6. Njameja (Igbo)	unknown	Njameja (Igbo)	Leaf

Table 2. Total polyphenol content and antioxidant activities spice extracts determined by ABTS, DPPH and ORAC assays

Spice	Total phenols	ABTS	DPPH	ORAC
Xylopia aethiopica	1536.39^{a}± 6.6	5978.5^{a} ± 3.1	12760.58^{b} ±40.34	379.3^{a} ±2.14
Tetrapleura tetraptera	1424^{b}± 68.1	5457.5^{b} ± 4.34	13038.9^{a} ±2.01	349^{b} ±4.02
Piper guineense (seed)	978.86^{c}± 8.62	1612^{d} ±1.38	6011.86^{c} ±17.18	316.74^{c} ±4.87
Piper guineense (leaf)	1038.7^{c} ±26.44	1635.58^{c} ±4.39	1165.6^{f} ±27.75	261^{d} ±12.21
Monodira myristica	353.86^{e}± 23.14	1073.4^{e} ±13.39	4777.4^{d} ±26.75	311.4^{c} ±10.09
Njameja leaf	869^{d} ±30.04	1043.74^{f} ±8.82	2398.4^{e} ±15.59	266.1^{d} ±4.88
Mean	1033.5 ±94.51	2800 504.86	6692 ±1129.9	313.94 ±10.49

Values are means of three replicates. A-F Means in the same column with different superscripts are significantly different (p<0.05).

Table 3. Pearson´s correlation coefficients of total phenolic and antioxidant activity measured by ABTS, DPPH and ORAC

	ABTS	DPPH	ORAC	TP
ABTS	1.000			
DPPH	0.932**	1.000		
ORAC	0.828**	0.926**	1.000	
TP	0.854**	0.693**	0.549*	1.000

*ABTS= antioxidant activity measured in ABTS assay, DPPH = antioxidant activity measured by DPPH assay, ORAC = antioxidant activity measured by ORAC assay, TP = total phenols, ** and * = correlation is significant at p<0.01 or p<0.05.*

Table 4. Probable identity of bacteria used for inhibition studies

Parameters	Probable organisms					
	Bacillus subtilis	***Staphylococcus***	***Escherichia coli***	***Salmonella***	***Lactobacillus***	***Klebsiella***
Colony morphology	Cream coloured Colonies with entire edges	Smooth glistening Yellow colonies	Large smooth glistening yellow colonies with entire edges	Pale coloured colonies with a smooth glossy surface	Cream coloured colonies	Opaque colonies that stretch when touched with a loop
Cell morphology	Motile rods in chains with central spores	Cocci in irregular structures	Short motile rods	Straight motile rods	Long slender non-motile noncapsulated and nonsporulated rods	Capsulated nonmotile nonsporulated short plump rods
Gram stain reaction	+ve	+ve	-ve	-ve	+ve	+ve
Spore stain reaction	+ve	-ve	-ve	-ve	-ve	-ve
Indole reaction	-ve	-ve	+ve	-ve	-ve	-ve
Coagulase test	+ve	+ve	-ve	-ve	-ve	-ve
Catalase test	+ve	+ve	+ve	-ve	-ve	-ve
Oxidase test	+ve	-ve	+ve	-ve	-ve	-ve
Sugar fermentation test						
Maltose	A	A	A/G	A/G	NT	A
Lactose	A	A	A	No fermentation	NT	A
Sucrose	A	A	No fermentation	No fermentation	NT	NT

NT= Not tested; A=Acid; -ve + negative; +ve + Positive; A/G= Acid and gas

Table 5. Inhibition of bacterial growth by some spices

Organism	Spices			
	A	B	C	D
Staphylococcus aureus	-	-	-	-
Lactobacillus	+	-	-	-
Escherichia coli	++	-	-	-
Salmonella	+	-	-	-
Klebsiella	++	-	-	-
Bacillus subtilis	+	-	-	-

A = Xylopia aethiopica; B = Tetrapleura tetraptera;
C = Piper guineense; D =Monodora myristica

Table 6. Morphological characteristics of fungal isolates used

Morphology of organism on potato dextrose agar	Morphology of organism on sabouraud agar	Microscopy	Probable organism
Black colony with yellow edges. Powdery surface colony which becomes black finally	White colonies with blackcentres which increased with time. The margins of the fungi were whitish	Septate hyphae Conidiophores formed which terminate in a swollen vesicle	Aspergillus species
Initially white and cotton like colonies which turns grey after three days with black globule structure present all over	Fluffy, whitish colonies that turned brown after three days	Large saclike structures that contain sporangiophores, connected to one another by septate hyphae	Rhizopus species

3.5 Inhibition of Select Bacteria by Spice Extracts

Table 5 shows the inhibitory effects of spice extracts on six bacterial species. Three out of the four extracts used for experiments were ineffective at the concentrations used for experiments. The extracts were found to be effective on two bacterial species: *Escherichia coli* and *Klebsiella*.

3.6 Probable Identity of Fungal Isolates Used for Inhibition Tests

Table 6 shows the probable identity of fungi used for inhibition tests. The two groups of fungi identified have strains that are associated with food spoilage. None of the spices inhibited these fungi at the concentration used for experiments (1 g/100 ml)

4. CONCLUSION

Quantitative evaluation of the spice extracts showed high levels of phenolic content and antioxidant activity. The spice extract with high phenolic compounds had higher antioxidant activities (ABTS, DPPH and ORAC), in the order *Xylopia aethiopica>Terapleura teraptera>Piper guineense* (leaf and seed) >*Monodora myristica*>Njameja leaf. A highly positive linear relationship was observed between phenolic content and antioxidant activity .This showed that phenolic compounds in the spice extracts contributed significantly to antioxidant activity. One important use of the spice extracts as the after cleansing of the women's womb has been remotely highlighted. Further investigation is required to discover the potential phenolic constituents and their applications. Quantitative studies should be carried out on antimicrobial properties especially *Xylopia aethiopica* which effectively inhibited the food borne pathogens at the concentrations used for experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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