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ANTIOXIDANT PROSPECTS OF THREE AFRICAN ETHNOMEDICINAL SPICES

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ABSTRACT

This study entails critical scrutiny of the antioxidant prospects of methanol extracts of *Xylopia aethiopica, Rhaphiostylis beninensis,* and *Piper guineenese* spices using the total phenolic content (TPC) and total flavonoid content (TFC) phytochemical assays coupled with the 2, 2- diphenyl-1- picrylhydrazyl (DPPH), Ferric ion reducing antioxidant power (FRAP), Phosphomolybdate (PMA), Nitric Oxide (NO) and Hydrogen peroxide scavenging activity (HPA) *in vitro* antioxidant assays. The extrapolated inhibitory concentration at half maximum (IC_{50}) and exhibitory concentration at half maximum (EC_{50}) values which denote the antioxidant efficacies of the spice extracts were statistically compared to that of their standard (ascorbic acid). Results revealed that the spices screened were rich in polyphenols and exhibited good antioxidant capabilities with *X. aethiopica* exhibiting the most outstanding attribute: TPC (16.69 mg GAE/g), TFC (11.90 mg RUE/g), DPPH (IC_{50} = 190.40 µg/mL), FRAP (EC_{50} =192.97 µg/mL), HPA (IC_{50} = 209.67 µg/mL), PMA (EC_{50} = 274.07 µg/mL and NO (IC_{50} = 290.90 µg/mL) assays. Nevertheless, *P. guineense* displayed the lowest antioxidant property in the TPC (4.28 mg GAE/g), TFC (1.72 mg RUE/g), DPPH (IC_{50} = 373.97 µg/mL), FRAP (EC_{50} =367.80 µg/mL), HPA (IC_{50} = 349.63 µg/mL), PMA (EC_{50} = 477.90 µg/mL) and NO (IC_{50} = 325.47 µg/mL) assays. Pearson's correlation analysis revealed significant linear relationships between the TPC, TFC, IC_{50}, and EC_{50} values of the plants. Thus, these results substantiate the therapeutic and prophylactic applications of the plants particularly, *X. aethiopica* in folklore medicine, and have led the path to their exploitation as novel sources of natural antioxidants for pharmaceutical and allied industries.

Keywords: *Xylopia aethiopica*; *Rhaphiostylis beninensis*; *Piper guineenese*; Natural antioxidant; Antioxidant activitv.

INTRODUCTION

Africa is endowed with notable medicinal plants which have been utilized as remedies for various free radical-related ailments over the years. This occurrence stems from the existent fact that two-thirds of plant species possess medicinal attributes and that; virtually all of these plants have excellent antioxidant potential (Bjelakovic *et al.,* 2013). Antioxidants help to mop up free radicals which are the principal initiators of inflammatory conditions hence, antioxidants may keep the body from various ailments resulting from exposure to free radicals. In addition, antioxidants minimize rancidity, attenuate the formation of toxic oxidation products, conserve nutritional quality, and prolong shelf life when added to foods (Jadhav *et al.,* 1996). In this regard, synthetic antioxidants have been an excellent tool, however, their applications in food systems of late have been restricted as a result of their reported harmful effects on valuable organs of the body such as the Liver and Lungs (Chowdhury *et al.,* 2011). Thus, the pursuit

of novel, harmless, and potent natural antioxidants to replace synthetic ones is ongoing. Accordingly, the inclusion of plant extracts derived from commercially available plant material, which are frequently utilized as a natural remedy or in culinary applications, is something that consumers pay close attention to while reading the label (Kozłowska *et al.,* 2022).

Phenolic compounds are widely dispersed in plants and are one of the plants' primary bioactive components with a variety of advantageous qualities such as cardioprotective, anticancer, immunomodulatory and antibacterial (Foss *et al,* 2022). Moreover, studies have indicated that they are major contributors to the antioxidant contents of plants (Tacouri *et al.,* 2013; Ngwoke *et al.* 2015). Furthermore, the antioxidant capacity of phenolic compounds is consistent with the number of renowned mechanisms of antioxidants such as free radical scavenging ability, hydrogen ion donating ability, singlet oxygen quenching capacity, metal ion chelating ability, and ability to serve as substrates for hydroxyl and superoxide radicals (Prakash and Kumar, 2011). Consequently, the antioxidant activity of phenolic compounds coupled with their prospective beneficial effects on human well-being has brought them to the limelight.

Spices are essential sources of various polyphenols with outstanding antioxidant capabilities (Shan *et al.,* 2005). Accordingly, exogenous antioxidants of plant origin are now being employed as components of nutraceuticals and food supplements; maximized to sustain human health and prevent disorders associated with oxidative stress (Evuen *et al.,* 2016). Thus, the ethnopharmacological and food preservative properties of *Xylopia aethiopica*, *Rhaphiostylis beninensis*, and *Piper guineense* spices have been exploited in various regions of Africa (Mishana *et al.,* 2000; Lasisi *et al.,* 2013; Ogueke *et al.,* 2018).

Xylopia aethiopica commonly known as *Ethiopian pepper*, is a member of the family, Annonaceae bearing odoriferous fruits. It grows virtually in all of the tropical and subtropical evergreen rainforests of Africa. Antimicrobial and various pharmacological activities such as anti-diabetic, hypoglycemic, and antioxidant activities have been reported for this plant (Fetse *et al.,* 2016). The woody climber, *R. beninensis* belonging to the family, Icacinaceae, is notable for its various medicinal attributes (Ofeimum and Ayinde, 2017; Evuen *et al.,* 2020). It thrives in the Southwestern region of Nigeria and the West African sub-region (Daziel, 1973). *Piper guineense* (West African Black pepper), a member of the Piperaceae family, is famous in West Africa for its reported anti-inflammatory, hepatoprotective, antimicrobial, and antioxidant properties (Besong *et al.,* 2016).

Given the ethnopharmacological reports of the spices and the ensuing upsurge in the quest for natural antioxidants that could serve as suitable alternatives to their synthetic counterparts, it is necessary to ascertain the general antioxidant activity of the said spices. In this respect, biochemical assays have proven to be the most authentic and readily available methods. However, because of multivariate responses encountered by a particular antioxidant in different testing systems, it is appropriate to employ various antioxidant assays to understand the mechanism of action of the bioactive principles involved. Moreover,

the utilization of diverse methods renders more detailed information concerning the antioxidant properties of the sample owing to significant variations in the choice of end-points and presentation of results (Viuda-Martos *et al.,* 2010). Therefore, this study investigated the antioxidant potential of methanol extracts of *Xylopia aethiopica*, *Rhaphiostylis beninensis,* and *Piper guineense* spice plants using *in vitro* phytochemical and antioxidant assays.

MATERIALS AND METHODS

Collection and Identification of Plant Samples

The spices, *Xylopia aethiopica* (Fruits), *Piper guineense* (seeds), and *Rhaphiostylis beninensis* (roots) were purchased from a local market in Oghara, Delta State, Nigeria, identified and authenticated by Dr. H.A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria. Specimens with voucher numbers, UBHx0348, UBHa0328, and UBHp0262 respectively were deposited in his herbarium.

Extraction

A large quantity of each spice sample was exposed to room temperature drying at 27.0 ± 2.0^{o}C for two weeks. Afterward, the spices were subjected to homogenization employing a warring mechanical blender to obtain dried, pulverized plant materials. Subsequently, extractions were carried out on each of the pulverized parts by maceration at 10 g/100 mL of methanol. The mixture was stirred, left for 72 hours, and filtered with a muslin cloth. Furthermore, the various extracts were concentrated in a vacuum to viscous slurry using a rotary evaporator (RotoVap RE-501, USA) at 40^{o}C. The concentrated extracts were weighed, stored in air-tight containers, and kept in a refrigerator (4^{o}C) until required for use.

Investigation of the Antioxidant Activities of Spice extracts.

DPPH Scavenging Activity

The antioxidant activity of the spice samples was assessed using the 2, 2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity in a method elucidated by Hasan *et al.,* (2006) and Alam *et al.,* (2008). A 0.004 g DPPH was dissolved in 100 mL of methanol and incubated in the dark for 10 min at ambient temperature to serve as a positive control. Three millilitres (3 mL) of the methanol-DPPH solution and 0.1 mL of graded levels (0 -500 µg/mL) of test samples were pipetted into test tubes, shaken steadily, and incubated for 15 min at 25 ± 3^{o}C. The absorbance was read immediately after incubation at 517 nm. Similarly, ascorbic acid was employed as the standard antioxidant at the same graded concentrations. The control was prepared devoid of the test sample or standard and absorbance was read at the said wavelength. The following equation was used for the computation of percentage inhibition of DPPH radical scavenging outcome, which was further employed in the development of a standard calibration curve and extrapolation of the IC_{50} value.

$$\%\ \text{DPPH Scavenging Effect} = \frac{A_{control} - A_{test}}{A_{control}} \times \frac{100}{1} \quad (1)$$

Where; A_{test} = absorbance of test sample or standard at 517nm

$A_{control}$ = absorbance of methanol-DPPH devoid of test sample or standard at 517 nm

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric-reducing antioxidant power of the spices was done using the "method described by Yildirim *et al.,* (2001)". A mixture in test tubes, consisting of 2 mL of the sample at graded concentrations (0-500 µg/mL), 2 mL of 0.2 M phosphate buffer (pH 6.6), and 2 mL of 1 % potassium ferricyanide, $K_3Fe(CN)_6$, was incubated for 20 min at 50°C. Thereafter, 10 % trichloroacetic acid was added to the mixture and centrifuged at 3000 g for 20 min. Subsequently, 2 mL of the supernatant, 2 mL of distilled H_2O and 400 µL of 0.1% fresh $FeCl_3$ were added and incubated at room temperature for 10 min for colour development, which was then monitored at 700 nm.

Similarly, ascorbic acid was employed as the standard antioxidant at the same graded concentrations. The control was prepared devoid of the test sample or standard. A phosphate buffer was used to zero the instrument and absorbance was read at the said wavelength. A standard calibration curve was developed and employed for the extrapolation of the IC_{50} value. Percentage inhibition was computed following the equation below:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times \frac{100}{1} \quad (2)$$

Where: $A_{control}$ = the absorbance of control devoid of test sample or standard at 700 nm. A_{test} = the absorbance of assay mixture including sample, (i.e. extract or standard) at 700 nm.

Total Antioxidant Capacity (Phosphomolybdate Assay)

This was evaluated by the phosphomolybdate method using ascorbic acid as a standard (Umamaheswari and Chatterjee, 2008). One millilitre (1 mL) of an equal amount of sample or standard (ascorbic acid) at graded concentrations (0-500 µg/ml), 1 mL of a reagent solution, which was a mixture of 0.6 M H_2SO_4, 28 mM sodium phosphate (Na_3PO_4) and 4 mM ammonium molybdate ($(NH_4)_2MoO_4$) were pipetted into test tubes, capped, incubated for 90 min at 95°C in a water bath and cooled. The absorbance was read at 765 nm against a reagent blank. The standard calibration curve was done and employed in the extrapolation of the EC_{50} value. Thus, the total antioxidant activity expressed as a percentage was computed from the equation hereunder:

$$\text{Total antioxidant effect (\%)} = [\frac{(A_{control} - A_{test})}{A_{control}} \times \frac{100}{1}] \quad (3)$$

Where: $A_{control}$ = absorbance of control devoid of test sample or standard at 765 nm.

A_{test} = the absorbance of assay mixture including sample, (i.e. extract or standard) at 765 nm.

Hydrogen Peroxide Scavenging Activity (HPA)

Scavenging activities of hydrogen peroxide (H_2O_2), by the plant extracts, were evaluated by the method of Jayaprakasha *et al.,* (2001). Four millilitres (4 mL) of each extracted sample at graded concentrations (0 - 500 µg/mL) and 600 µL of 4 mM H_2O_2 solution were pipetted into test tubes, permitted to stand for 10 min and the absorbances were read at 230 nm against a reagent blank. Thus, the H_2O_2 scavenging activities of the spice samples were then estimated as shown below:

$$H_2O_2 \text{ scavenging activity (\%)} = [\frac{(A_{control} - A_{test})}{A_{control}} \times \frac{100}{1}] \quad (4)$$

Nitric oxide radical scavenging assay

The method of Garrat (1964) was used to determine the nitric oxide radical scavenging activities of the spices. Two millilitres (2 mL) of 10 mM sodium nitroprusside prepared in phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of the spice extract at various concentrations ranging from 0 to 500 µg/mL and ascorbic acid at various concentrations ranging from 0 to 500 µg/mL. The mixture was incubated at 25°C. After 150 min, 0.5 ml of the incubated solution was withdrawn and mixed with 0.5 ml of Griess reagent [1.0 ml sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthyl ethylene diamine dihydrochloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min. Thereafter, absorbance was measured at 540 nm using a spectrophotometer (Shimadzu UV-1800). The nitric oxide radical scavenging activities of the extracts were reported as percentage inhibition and calculated using Equation 2.

Estimation of Total Phenolic Content (TPC)

The total phenolic content was assessed by the spectrophotometric method described by Kim *et al.* (2003). One millilitre (1 mL) of the test sample (1 mg/mL) and 1 mL of Folin-ciocalteu phenol reagent (1:10 dilution) were mixed and incubated for 5 min. Ten millilitres (10 mL) of sodium trioxocarbonate (iv) (Na_2CO_3) solution (7.0 % w/v) and 13 mL of distilled H_2O were added to the mixture, and stirred properly and incubated for 90 min at 23°C. The absorbance was read instantaneously at 750 nm against a reagent blank.

A standard calibration curve using gallic acid as the standard phenolic compound was prepared following the aforesaid assay to evaluate TPC in triplicate determinations. The TPC of the spices was determined from the calibration line, $y = 0.002x - 0.0824$; $R^2 = 0.9586$ plotted using Gallic acid as a standard, and results were expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

Estimation of Total Flavonoid Content (TFC)

Total flavonoid content was assessed following the "method of Park *et al.*, (2008)". One millilitre (1 mL) of the test sample (1 mg/mL) and 150 µL of 0.3M aluminium trichloride hexahydrate ($AlCl_3.6H_2O$) were pipetted and incubated for 5 min. 1 mL of 1 M NaOH was added to the resulting mixture, and the absorbance was read immediately at 506 nm against a reagent blank. A standard calibration curve using rutin as the standard flavonoid compound was prepared following the aforesaid assay to evaluate TFC in triplicate determinations. The TFC of the spices was determined from the calibration line, $y = 0.001x + 0.1277$; $R^2 = 0.9893$ plotted using Rutin as a standard, and results were expressed as milligrams of rutin equivalents (RUE) per g of dried sample.

Data Analysis

Data obtained from the antioxidant activities of the spices were analyzed with the help of a computerized GraphPad prism 8.0.2 (263) software to determine the IC_{50} and EC_{50} values of the spices. The data were presented as Mean ± SEM (standard error of the mean) from three separate observations. Pearson's correlation coefficient (r) was also used to evaluate correlations between the results of different antioxidant assay methods.

The one-way analysis of variance (ANOVA) was used to verify any variation

in antioxidant activities of the spices consequent upon the usage of different antioxidant assay schemes. The statistical package (SPSS 21.0) was employed for the ANOVA. Mean values of various groups were significantly compared by Tukey's Multiple Range Test and a probability of $p< 0.05$ was considered significant.

RESULTS

3.1 Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) of the spice extracts

The total phenolic and total flavonoid contents of the spice extracts ranged from 4.28mg GAE/g to 16.69mg GAE/g and 1.72mg RUE/g to 11.90mg RUE/g respectively. Moreover, the TFC and TPC of the three spices were significantly different ($p<0.05$) from each other. *Xylopia aethiopica* and *P. guineense* had the highest and lowest TFC and TPC values of the three spices respectively.

Table 1: Total Flavonoids and Total Phenolic Contents of methanol extracts of *R. beninensis, P. guineense* and *X. aethiopica* spice extracts

Spices	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg RUE/g)
R. beninensis	10.20 ± 0.10^{a}	6.80 ± 0.26^{d}
P. guineense	4.28 ± 0.23^{d}	1.72 ± 0.18^{b}
X. aethiopica	16.69 ± 0.23^{k}	11.90 ± 0.37^{f}

Values are expressed as mean ± standard error of mean (X ± S.E.M*)* in triplicate. *Values with different letters along the same column are significantly different (p<0.05).*

IC_{50} and EC_{50} values of the spice extracts

The extrapolated inhibitory concentration at half maximum (IC_{50}) and exhibitory concentration at half maximum (EC_{50}) values for the various spices in DPPH and FRAP assays respectively, differ significantly ($p<0.05$) with *X. aethiopica* recording lowest values (higher antioxidant properties) followed by *R. beninensis* and then *P. guineense* compared with the standard (ascorbic acid). Similarly, the IC_{50} values for *X. aethiopica* in the Hydrogen Peroxide Assay (HPA), Nitric oxide (NO) assay and its EC_{50} value for the Phosphomolybdate Assay (PMA) relative to those of *P.guineense* and *R.beninensis* spices, showed marked significant differences ($p<0.05$). Moreover, *P.guineense* and *R.beninensis* spices showed no significant differences ($p>0.05$) in their antioxidant activities in the said assays. Generally, following the estimated IC_{50} and EC_{50} values for the various spice extracts compared with that of the standard, the antioxidant activities of the spices in increasing order of magnitude were as follows; *P. guineense* < *R. beninensis* < *X. aethiopica* < Ascorbic acid.

Table 2: IC_{50} and EC_{50} values for *R. beninensis, P. guineense* and *X. aethiopica* spice extracts relative to Ascorbic acid

Spices and Standard	IC_{50} of DPPH (µg/mL)	EC_{50} of FRAP (µg/mL)	IC_{50} of HPA (µg/mL)	EC_{50} of PMA (µg/mL)	IC_{50} of NO (µg/mL)
Ascorbic acid	100.90 ± 22.82^{a}	110.63 ± 4.84^{a}	119.33 ± 17.47^{a}	188.63 ± 2.38^{a}	279.60 ± 0.30^{a}
P. guineense	373.97 ± 6.06^{b}	367.80 ± 17.93^{b}	349.63 ± 0.29^{b}	477.90 ± 48.24^{b}	325.47 ± 0.90^{b}
X. aethiopica	190.40 ± 5.26^{c}	192.97 ± 1.78^{c}	209.67 ± 1.10^{c}	274.07 ± 2.68^{c}	290.90 ± 0.30^{c}
R. beninensis	274.63 ± 7.69^{d}	262.43 ± 3.32^{d}	333.33 ± 16.68^{b}	411.13 ± 31.04^{b}	324.57 ± 0.03^{b}

Values are expressed as mean ± standard error of mean (X ± S.E.M*)* in triplicate. *Values with different letters along the same column are significantly different (p<0.05).*

To corroborate the results from the IC_{50} and EC_{50} values for the spices in different antioxidant assays (Table 2), the percentage inhibitions exhibited by various concentrations (0-500 µg/mL) of the spice extracts in the various antioxidant assays were graphically compared to that of their assay standard, ascorbic acid (Figures 1-5).

Pearson's correlation between total phenolic, total flavonoid contents, IC_{50} and EC_{50} values of the spices in various antioxidant assays

Correlation analysis between TPC, TFC, IC_{50} and EC_{50} values of the spices for the antioxidant assays explored (DPPH-FRSA, PMA, HP-FRSA, NO-FRSA) showed that there were significant linear relationships between the IC_{50} and EC_{50} values of the spices. TPC in the spice samples had significant and negative correlations with their IC_{50} values; DPPH FRSA ($r = -0.989$, $p<0.01$), HP FRSA ($r = -0.828$, $p<0.05$), NO FRSA ($r = -0.889$, $p<0.01$) and EC_{50} values: PMA ($r = -0.814$, $p<0.01$), FRAP ($r = -0.976$, $p<0.01$). In the same vein, there were also significant and negative correlations between their TFC and IC_{50} values; DPPH FRSA ($r = -0.988$, $p<0.01$), HP FRSA ($r = -0.800$, $p<0.01$), NO FRSA ($r = -0.873$, $p<0.01$) and TFC and EC_{50} values; FRAP ($r = -0.979$ $p<0.01$), PMA ($r = -0.837$, $p<0.01$) respectively. Moreover, there was a strong significant correlation ($r = 0.994$, $p<0.01$) between values for the TPC and TFC contents of the spices.

Table 3: Pearson's Correlation Coefficient of Total Phenolic Content, Total Flavonoid Content with IC_{50} and EC_{50} values of the spices in various antioxidant assays.

	DPPH-FRSA	PMA	FRAP	HP-FRSA	NO-FRSA	TPC	TFC
DPPH-FRSA	1.000						
PMA	0.866[b]	1.000					
FRAP	0.988[b]	0.895[b]	1.000				
HP-FRSA	0.764[a]	0.542	0.765[a]	1.000			
NO-FRSA	0.846[b]	0.525	0.842[b]	0.845[b]	1.000		
TPC	-0.989[b]	-0.814[b]	-0.976[b]	-0.828[a]	-0.889[b]	1.000	
TFC	-0.988[b]	-0.837[b]	-0.979[b]	-0.800[b]	-0.873[b]	0.994[b]	1.000

PMA: Phosphomolybdate assay, FRAP: Ferric reducing antioxidant power, HP-FRSA: Hydrogen peroxide free radical scavenging activity, NO-FRSA: Nitric oxide free radical scavenging activity, OH-FRSA: Hydroxyl free radical scavenging activity, DPPH-FRSA:2,2-Diphenyl-1-picrylhydrazyl free radical scavenging activity, [a] and [b] = correlation is significant at $p < 0.05$ or $p < 0.01$.

DISCUSSION

Total phenolic and total flavonoid contents of the spices

In the present study, *X. aethiopica* had the highest total phenolic and total flavonoid contents of the three spices, hence, *X. aethiopica* may serve as a better source of natural antioxidants compared with the two other spices. These results are consistent with the findings of a previous report where the total phenolic and total flavonoid contents of *X. aethiopica* were maximal compared with that of *Piper guineensis* and *Ocimum basilicum* spices (Omoba *et al.*, 2019).

A review of literature revealed that there were no reports for the total phenolic and total flavonoid contents of *R. beninensis* root. However, data obtained for the total phenolic content and total flavonoid content of *P.guineense* and *X. aethiopica*, are in line with reports from earlier studies (Adefegha and Oboh, 2012; Etim *et al.*, 2013). Adefegha and Oboh (2012) reported that mean concentrations of total phenolic and total flavonoid contents for *P. guineense* seeds were 4.6±0.4mg GAE/g and 2.5±0.2mg RUE/g respectively. Similarly, Etim *et al.*, (2013) reported that mean concentrations of total phenolic and total flavonoid contents for *P. guineense* seeds were 1.16±0.17mg GAE/g and 1.28±0.47mg RUE/g respectively. The said

values are comparable to 4.28±0.23mg GAE/g and 1.72±0.18mg RUE/g respectively obtained in this study. On the other hand, Yusuf *et al.,* (2018) obtained a value of 15. 98±0.03mg GAE/g for the total phenolic content of *X.aethiopica* fruits which is consistent with 16. 69 ±0.23mg GAE/g that was obtained in this study.

Contrary to the above reports, a value of 39.10 ±0.26mg GAE/g reported for the total phenolic content of *X. aethiopica* by Gbadamosi and Kalejaye (2017) is not in line with 16. 69 ±0.23mg GAE/g that was obtained in this study. In the same vein, Owoade *et al.,* (2017) reported a value of 37.15 ±2.62mg GAE/g as the total phenolic content of *P. guineense* seeds which does not also conform to 4.28 ±0.23mg GAE/g obtained in this study. However, the reasons for the discrepancies in values could be differences in solvents used for extraction, genetic factors, degree of maturation, and the duration of storage of the spices.

Antioxidant activity of the spices

The ability of the investigated methanol extracts to act as donors of hydrogen atoms or electrons in the transformation of DPPH radical into its reduced form is attendant to its degree of discolouration and scavenging capacity (Phatak *et al.,* 2015). Moreover, the inverse relationship between the IC_{50} or EC_{50} values and the antioxidant potency of a plant extract suggests that the lower the IC_{50} or EC_{50} values, the higher the antioxidant capacity of the plant and vice versa. Thus, the comparative IC_{50} values for *P. guineense, X. aethiopica* and *R. beninensis* spices respectively relative to the standard ascorbic acid, revealed that among the spices, *X. aethiopica* had the best hydrogen-donating ability to scavenge DPPH radical as evident by its lowest IC_{50} value. Antioxidants with good DPPH radical scavenging activity could donate hydrogen to free radicals and form non-radical species; bringing the propagating stage of lipid peroxidation to a halt (Bamforth *et al.,*1993). Interestingly, the extract of *X.aethiopica* which displayed significant DPPH scavenging activity, also had a higher quantity of phenolic contents; indicating a linear correlation in its antioxidant ability. This observation is in line with earlier studies (Tacouri *et al.,* 2013; Gbadamosi and Kalejaye, 2017; Ojimelukwe and Ukom, 2017) suggesting that its high phenolic content contributed to the anti-radical activity.

The reducing power of a plant extract is naturally associated with the existence of reductants that exhibit antioxidant action by breaking the free radical chains through their electron-donating action (Rahman *et al.,* 2015). Thus, reducing power is also an indicator of a plant's antioxidative activity. Ferric ion reducing antioxidant power assay revealed that the three spice extracts showed a good reducing power capacity, which was concentration-dependent. This result was further corroborated by the IC_{50} and EC_{50} values of the various spice extract relative to standard ascorbic acid with *X. aethiopica* possessing the highest ferric reducing power thus, the best antioxidant potential.

Reports have shown that Hydrogen peroxide can cross cell membranes rapidly; the moment it gets inside the cell, H_2O_2 can probably react with transition metals such as Fe^{2+} and Cu^{2+} to form hydroxyl radicals causing lipid peroxidation and damage to the cell membranes and DNA (Jan *et al.,* 2017). In a bid to attenuate the toxicological consequence of H_2O_2, the scavenging of H_2O_2 is essential for the protection of living systems. The H_2O_2 scavenging assay shows

that the inhibitory activity of each extract (conversion of H_2O_2 to H_2O) increased with the increasing concentration of sample extracts. *Xylopia aethiopica* exhibited the highest percentage of inhibition of the three spices. Moreover, the estimated IC_{50} values for various spice extracts comparable to that of the standard (ascorbic acid) revealed that; *X.aethiopica* exerted the highest antioxidant capacity to scavenge H_2O_2. It is therefore reasonable to say that its high contents of phenolics with underlying antioxidant properties are the reasons for this outcome.

Nitric oxide is a very powerful oxidizing radical that leads to tissue damage in numerous diseased conditions in humans and experimental animals (Valko *et al.*, 2007). Though all the spice extracts exhibited nitric oxide scavenging ability, the scavenging activity of *X. aethiopica* was maximum. The observed nitric oxide scavenging by extracts of the spices could have been achieved by decreasing the amount of nitrite produced from the breakdown of sodium nitroprusside. Moreover, the antiradical activity of polyphenols in the spice samples is dependent on the molecular framework, the commutation of the hydroxyl groups, the presence of phenolic hydrogen, and the prospect of stability of the resulting nitric oxide radicals via donation of hydrogen or increase in electron delocalization (Erkan *et al.*, 2011).

Evaluation of the antioxidant capacity obtained by the Phosphomolybdate assay/Total antioxidant content (TAC) determination assay, revealed that *X. aethiopica* exhibited the highest phosphomolybdenum reduction followed by *R. beninensis* and then, *P. guineense* in a concentration-dependent manner. This reduction capacity was sustained by the estimated IC_{50} values of the spices for the TAC assay having a similar trend in antioxidant capacities. These results may be explained by the fact that the transfer of electrons/hydrogen from antioxidants depends on the structure of the antioxidants coupled with a special preference for the hydroxyl groups of flavonoids (El-sayed, 2009). More so, phenolic compounds are hydrogen donors; making them good antioxidants.

Pearson's correlation between total phenolic, total flavonoid contents, IC50 and EC_{50} values of the spices in various antioxidant assays

Following the work of Thaipong *et al.*, (2006), Pearson's correlation coefficient would be positively high if $0.61 \leq r \leq 0.971$ and negatively high if $-0.61 \leq r \leq -0.97$. Samples exhibiting a lower IC_{50} or EC_{50} value usually gave the highest antioxidant activity. So, a good correlation between IC_{50} or EC_{50} values of the spices with their TPC and TFC will be given in negative and high correlations. The meaning of the previous statement is that; an increase in the TPC and TFC contents of the spices brought about a similar effect (increase) in their antioxidant activities, which was revealed by a lower IC_{50} value. Therefore, the good correlation between TPC and TFC with IC_{50} or EC_{50} values will be significant and negative.

Results showed that TPC in the spice samples had significant and negative correlations with their IC_{50} or EC_{50} values; corroborating the saying that; there exists an inverse relationship between the IC_{50} or EC_{50} values of plant samples and their antioxidant capacity thus, to their phenolic contents (Moukette *et al.*, 2015). In addition to the above relationships, the close

correlation between TPC and TFC shows that phenolic and flavonoid compounds are the major contributors to the antioxidant activities of the spices by DPPH-FRSA, HP-FRSA, NO-FRSA, PMA and FRAP assays, hence, the DPPH-FRSA, HP FRSA, NO FRSA and PMA FRAP assay results of the spices can be indirectly predicted by investigating their TPC and TFC. Several authors have also reported significant and negative correlations between IC_{50} and EC_{50} values of plant samples and their total phenolic contents (Kusmardiyani *et al.*, 2016; Kanmaz and Saral, 2017; Fidrianny *et al.*, 2018).

CONCLUSION

Although; methanol extracts of *X. aethiopica* had the highest antioxidant prospect in this study, the two other spice extracts, *R. beninensis*, and *P. guineense*, exhibited considerable antioxidant capacities. Noticeable correlations among IC_{50} and EC_{50} values of the spices in various assays explored indicate that the antioxidant assays chosen in the present study are practicable and complementary to their antioxidant abilities in normal experimental conditions. These results show that the three spices are natural antioxidants eligible for employment as ingredients in the production of drugs and chemical components for combating free radical-related ailments in humans and oxidation in food substances. Accordingly, the fountain of natural antioxidants from African medicinal plants would be augmented. Nevertheless, further studies targeted at isolating and characterizing the bioactive components of the spices especially, *X. aethiopica*, are recommended.

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FIGURES

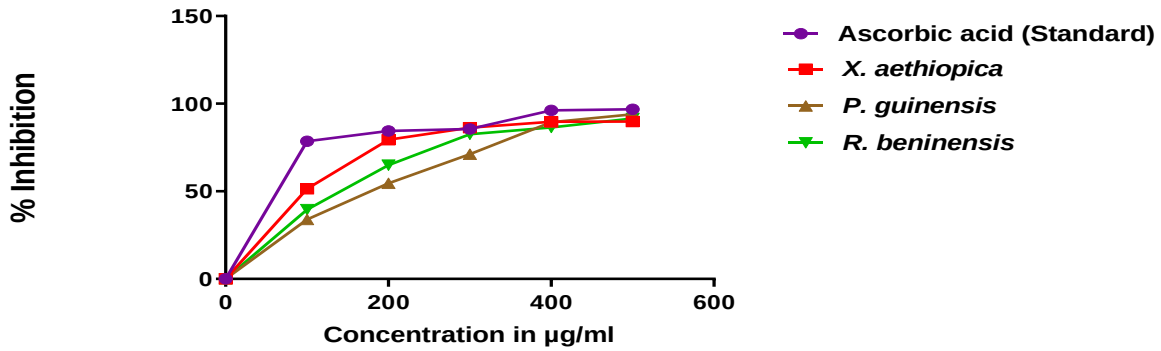

Fig. 1: Percentage inhibition of 2, 2- diphenyl-1- picrylhydrazyl by three spice extracts relative to a standard.
Data were expressed as Mean ± Standard Error of Mean (SEM) of triplicate values (n = 3). Values differ significantly at $p < 0.05$. The free radical scavenging activities of the spices were concentration-dependent. Compared to Ascorbic acid (the standard), *X. aethiopica* (red line) exhibited the highest percentage inhibition of the DPPH radical among the spice extracts.

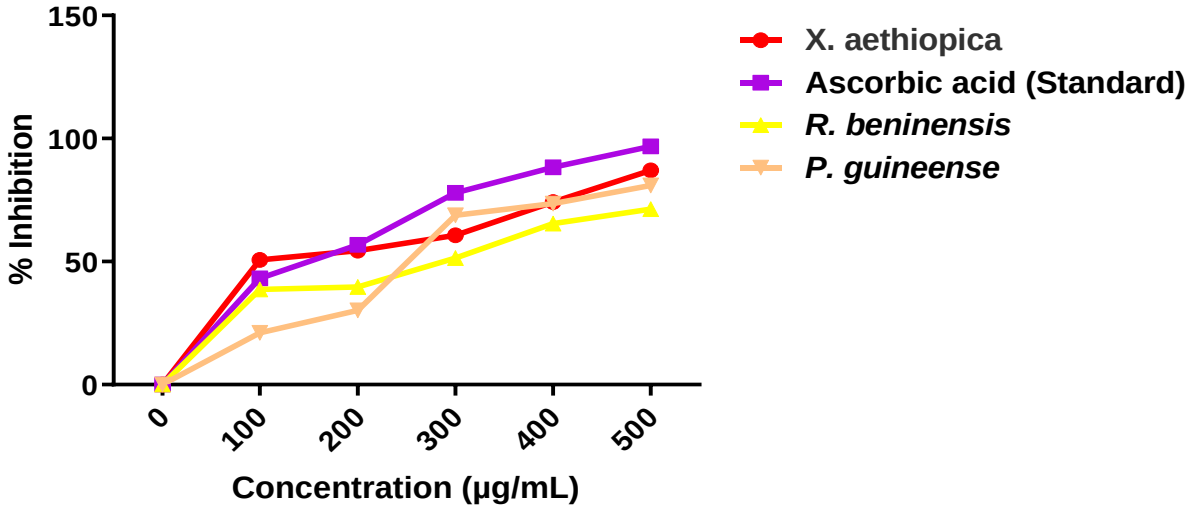

Fig. 2: Total antioxidant contents of three spice extracts relative to a standard.
Data were expressed as Mean ± Standard Error of Mean (SEM) of triplicate values (n = 3). Values differ significantly at $p < 0.05$. The antioxidant activities of the spices increased with increasing concentrations. Among extracts of the spices, *X. aethiopica* (red line) displayed the best capacity to *r*educe Mo^{6+} to Mo^{5+} compared with Ascorbic acid (Standard)

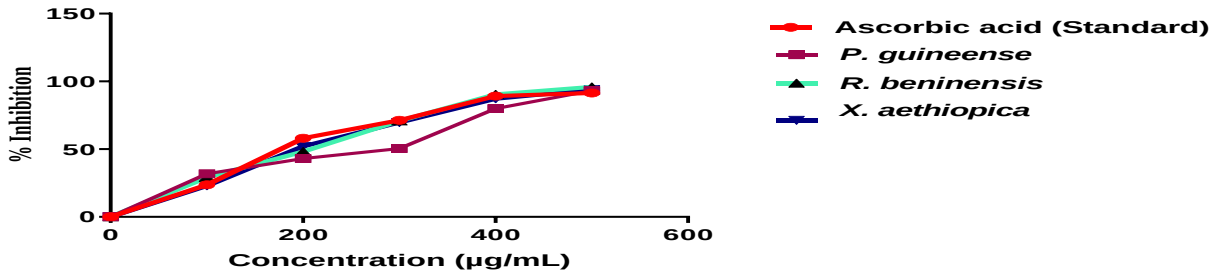

Fig. 3: Ferric Reducing Antioxidant Power of three spice extracts relative to a standard.

Data were expressed as Mean ± Standard Error of Mean (SEM) of triplicate values (n = 3). Values differ significantly at $p < 0.05$. The antioxidant activities of the spices were concentration-dependent.The propensity of *Xylopia aethiopica* (blue line) and *Rhaphiostylis beninensis* (turquoise line) spice extracts to reduce potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}) was comparable to that of Ascorbic acid (Standard).

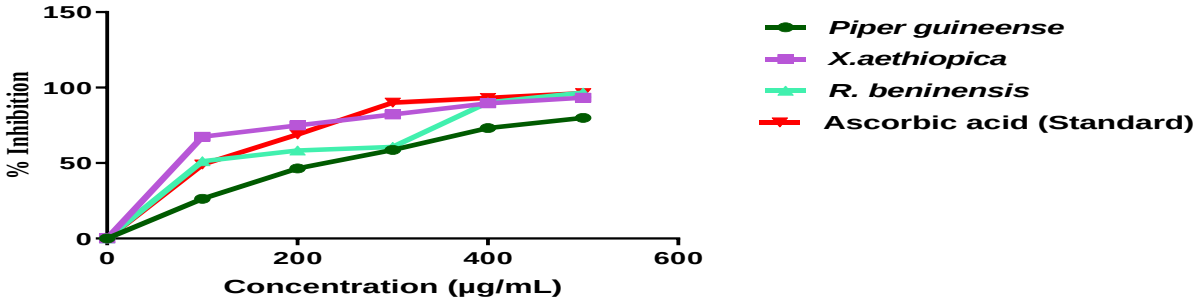

Fig. 4: Hydrogen peroxide scavenging activities of three spice extracts relative to a standard.
Data were expressed as Mean ± Standard Error of Mean (SEM) of triplicate values (n = 3). Values differ significantly at $p < 0.05$. The free radical scavenging activities of the spices were concentration-dependent. Among extracts of the spices, *Xylopia aethiopica* (purple line) exhibited the highest capacity to scavenge hydrogen peroxide**.**

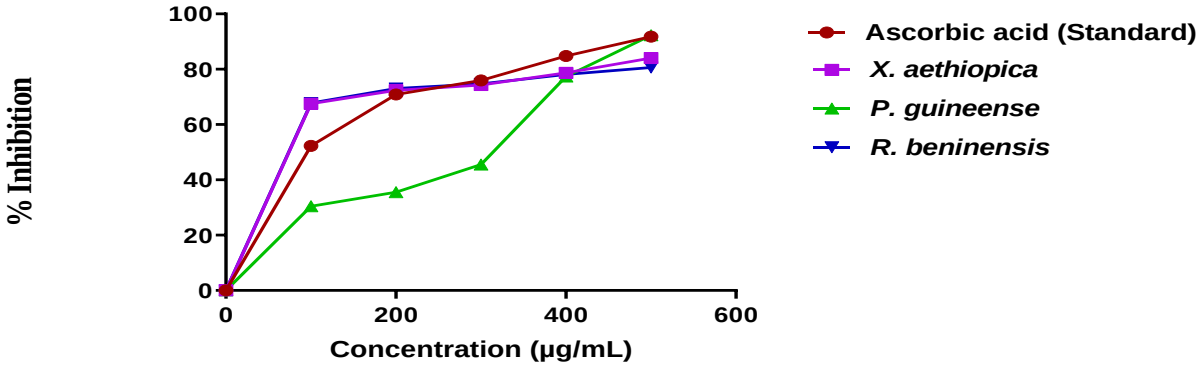

Fig. 5: Nitric oxide scavenging activities of three spice extracts relative to a standard.

Data were expressed as Mean ± Standard Error of Mean (SEM) of triplicate values (n = 3). Values differ significantly at $p < 0.05$. The free radical scavenging activities of the spices were concentration-dependent. Among extracts of the spices, *Xylopia aethiopica* (purple line) exhibited the highest capacity to scavenge nitric oxide**.**