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Full Length Research Paper

Phenolic content and total antioxidant capacity of local spices in Nigeria

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The use of natural antioxidants in the preservation of food is preferred over synthetic compounds. Spices show potential health benefits as they possess antioxidant activity. This study was designed to screen the antioxidant properties of *Aframomum sceptrum, Xylopia aethiopica, Monodora myristica* and *Allium sativum* using four different extractive solvents (water, ethanol, methanol and heated water). Phenolic content, total antioxidant capacity, reducing power and ascorbate oxidase activity were assayed. Methanolic extract of these spices showed relatively higher amounts of total phenolics than the other extracts. Total antioxidant capacity of the heated water extract of all the spices investigated was significantly higher than the levels obtained for all other extracts. The results further revealed that the reducing powers of spices were directly proportional to concentrations and their ascorbate oxidase activities were comparable. Local spices therefore possess antioxidant properties that can be used by food and pharmaceutical industries as potential sources of natural antioxidants.

Key words: *Aframomum sceptrum, Allium sativum,* antioxidant capacity*, Monodora myristica,* phenolic content*, Xylopia aethiopica.*

INTRODUCTION

Science centuries, spices have been utilized to enhance the flavoring and nutritive potentials of human food. The use of spice as food additives has been an aged long practice carried out by different people in many part of the world (Ifesan et al., 2006; Giese, 1994). Some spices and plant materials not only provide flavor, aroma and retard microbial growth in foods, but these are also beneficial in prevention of some off-flavor development as they retard oxidative degradation of lipids thereby improving quality and nutritional value of food (Politeo et al., 2006; Javanmardi et al., 2003; Rice-Evans et al., 1996; Landry, 1995; Giese, 1994). The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues (Hirasa and Takemasa, 1998).

Antioxidants are known to protect food quality by arresting or repressing free radical oxidation of fats and oils and the resulting off flavor colour (Buck, 1991). Synthetic antioxidants have been commonly used to prevent undesirable oxidation in many foods. Presently, antioxidants from natural sources are preferable to synthetic additives especially because butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) are suspected to have carcinogenic activity (Ozean, 2003) and epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancers (Renaud et al., 1998; Temple, 2000). The concern for natural additives for the stabilization of fat containing food stuffs has been increased.

Aframomum sceptrum (Family-Zingiberaceae, local name: Urioma/Ataiko), *Monodora myristica* (Family-Annonaceae, local name: Erhie (African nutmeg) and *Xylopia aethiopica* (Family-Annonaceae, local name: Uda) are three local spices commonly used to enhance

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flavor, aroma and palatability in cooking in the Southern part of Nigeria, particularly by the Urhobos, Itsekiris and Ijaws of Delta State. Apart from their use in cooking, spices have been repoted as an ingredient in the preparation of native medicinal concoctions with ethanol as a base solvent (George et al., 2010). Garlic (*Allium sativum*) extracts possessed interesting antioxidant properties and the *Allium* spices were among the most studied vegetables and have aroused great interest for food industries (Benkeblia, 2005).

The objective of this study was to screen the antoxidant properties of these spices and evaluate them as potential sources of natural antioxidant.

MATERIALS AND METHODS

Chemical and reagents

Folin-Ciocalteu phenol reagent**,** gallic acid were purchase from Sigma-Aldrich, Germany. Anhydrous Sodium carbonate, Trichloroacetic acid (TCA), Potassium ferricyanide, anhydrous ferric chloride, ascorbic acid and all other chemicals were of analytical grade and procured from BDH Chemical Laboratory, England, United Kingdom.

Plant materials

A. sceptrum (Atiako), *Monodora myristica* (African nutmeg), *Xylopia aethiopica* (Uda) and *A. sativum* (Garlic) were purchased from as local market in Abraka, Delta State, Nigeria. The spices were identified at the Department of Botany, Delta state University, Abraka, Delta State.

Extraction

The spices were sun dried why not at constant known temperature in oven to a constant weight for two weeks and were crushed into fine particles using Waring blender for 3 min at high speed. One gram of the powdered spice material was extracted with 10 ml of the respective solvent (water, ethanol, and methanol) expect with the hot water extract. Extraction was allowed to stand for 72 h, filtered and the filtrates was used for the following analysis:

Total phenolic compound analysis

Total phenolics were determined with the Folin-Ciocalteu reagent using the method of Macdonald et al. (2001). To 50 ul of each sample (three triplicate), 2.5ml 1/10 dilution of Folin-Ciocalteu's reagent and 2ml of Na_2CO_3 (7.5%w/v) were added and incubated at 45ºC for 15min. The absorbance of all samples was measured at 765 nm using a Uniscope UV-75 Series spectrophotometer. Calibration curve was prepared by mixing ethanolic solution of gallic acid and results were expressed as milligramme of gallic acid equivalent per gram of dry weight (mgGAE/g dw).

Reducing power assay

Two milliliters of extract were added to 2.5 ml of 1% potassium ferricyanide and the mixture incubated at 50ºC for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, centrifuged at 650 × g for 10 min. 2.5 ml of the supernant was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and the absorbance was measured at 700 nm (Oyaizu, 1986). Higher absorbance of the reaction mixture indicates greater reductive potential which is calculated as follows:

$$RP = \left[\frac{A_m}{A_b} - 1\right] x\,100$$

A_m = absorbance of reaction mixture
A_b = absorbance of blank mixture (distilled water instead of extract)

Assay of ascorbate oxidase activity

The sample was mixed [1:5 (v/v)] with phosphate buffer (0.1M/pH 6.5) and centrifuged at 3000 g for 15 min at 5ºC. The supernatant obtained was enzyme source. 0.1 ml of the enzyme extract was added to 30 ml of the substrate solution (8.8 mg ascorbic acid in 300 ml phosphate buffer, pH 5.6) and the change in absorbance was measured at 265 nm for every 30 s for a period of 4 min. enzyme activity is expressed as U/ml (Vines and Oberbacher, 1965).

Determination of total antoxidant capacity

The total antioxidant capacity was determine using commercially available kit as supplied by Randox Laboratories Ltd., Antrim, United Kingdom.

Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compound by Duncan's Multiple Range Test (DMRT). Values were considered statistically different when at $P < 0.05$.

RESULTS

Total phenolic content

Figure 1 shows the total phenolic content of *A. sceptrum, X. aethiopica, M. myristica* and *A. sativum* in four extracting solvents (water, ethanol, methanol and heated water). The results revealed that methanol extracted maximum phenolics for all the spices except *A. sativum*. Total phenolic content extracted by water is as follows; *Xylopia a. > A. sceptrum. > A. sativum > M. myristica.* Results for ethanol extraction indicated that *A. sceptrum* contained maximum phenolics (6.60±0.20 mg/g in) followed by *M. myristica* (2.93±0.42 mg/g), *X. aethiopica*. (0.42±0.02 mg/g) and *A. sativum* (0.41±0.12 mg/g) in terms of GAE, respectively. Figure 1 also showed that the amount of total phenolic content obtained using heated water as an extracting solvent is in the order of *M. myristic a > X. aethiopica > A. sativum > A. sceptrum*.

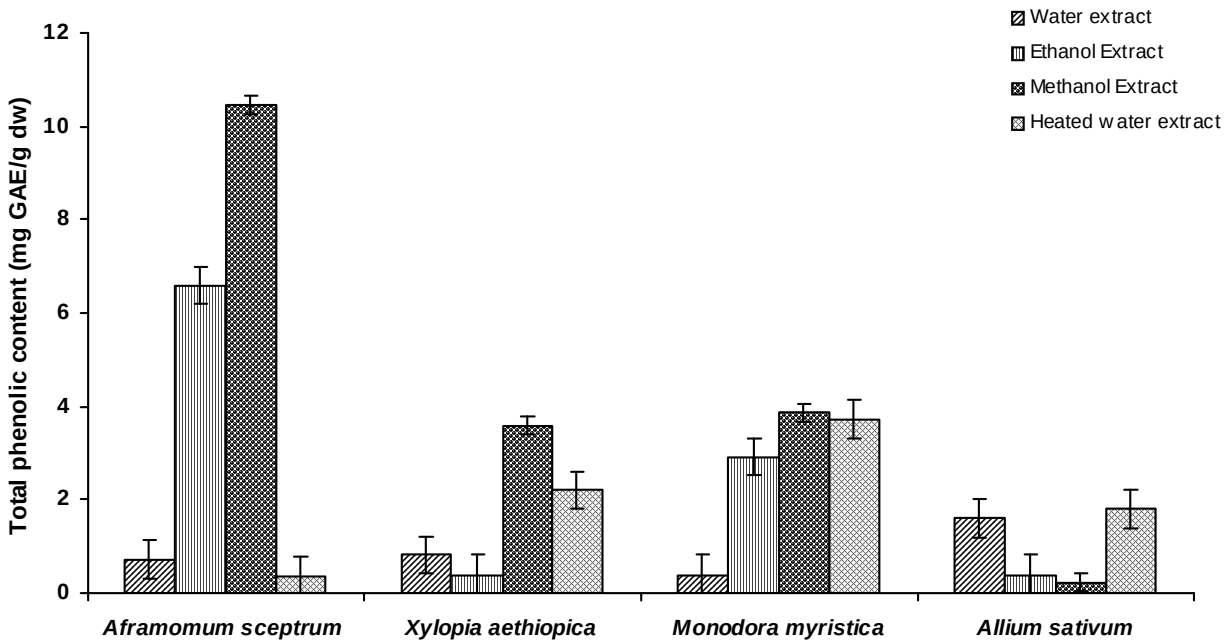

Figure 1. Total phenolic content of *Aframomum sceptrum*, *Xylopia aethiopica*, *Monodora myristica* and *Allium sativum* extracts using water, ethanol, methanol and heated water. Bars indicate mean±SD of triplicate determinations.

Table 1. Total Antoxidant capacity of *Aframomum sceptrum*, *Xylopia aethiopia*, *Monodora myristica* and *Allium sativum* extracts using water, ethanol, methanol and heated water.

Spices	Water	Ethanol	Methanol	Heated water
Aframomum sceptrum	2.43±0.19[a]	2.28±0.14[a]	3.04±0.08[b]	2.40±0.32[a]
Xylopia aethiopica	1.45±0.07[a]	0.12±0.04[b]	2.51±0.13[c]	3.11±0.22[d]
Monodora myristica	2.57±0.16[a]	2.70±0.26[a]	0.48±0.03[b]	3.02±0.74[c]
Allium sativum	1.61±0.04[a]	2.54±0.13[b]	1.56±0.12[a]	3.35±0.54[c]

Values are expressed as mean±SD of triplicate determinations. Values bearing same superscript letter on same row do not differ significantly at P> 0.05.

Antioxidant capacity

Table 1 indicates the total antioxidant capacity in mmol/L of the four spices investigated. Significant difference (P<0.05) was observed in total antoxidant capacity of the methanolic extract of *A. sceptrum* (3.04±0.08 mmol/L) as compared to water extract (2.43±0.19 mmol/L), ethanol extract (2.28±0.14) and heated water extract (2.40±0.32 mmol/L). Although, the mean total antoxidant capacity of *A. sceptrum* extracted with water, ethanol and heated water were comparable (P>0.5). Significant difference exits between all the extracts of *X. aethiopica* however, the heated water extract recorded the highest total antioxidant capacity (3.11±0.22 mmol/L) while the ethanol extract, the lowest, (0.12±0.04 mmol/L).

No significant difference was observed between the ethanolic and water extract with respect to the amount of total antoxidant capacity of *M. myristica* although both (water and ethanol) extract differed significantly from the methanolic and heated water extract. The table also revealed that the total antoxidant capacity of heated water extract of *A. sceptrum* is significantly elevated (3.35±0.54 mmol/L) as compared with the water (1.61±0.4 mmol/L), ethanol (2.54±0.13) and methanolic extract (1.56±0.12 mmol/L), respectively. An overall increase in the total antioxidant capacity of heated water extract of the spices except for *A. sceptrum* was observed.

Ascorbate oxidase activity

Figure 2 shows that ascorbate oxidase activity of *A. sceptrum*, *X. aethiopica* and *M. myristica* were

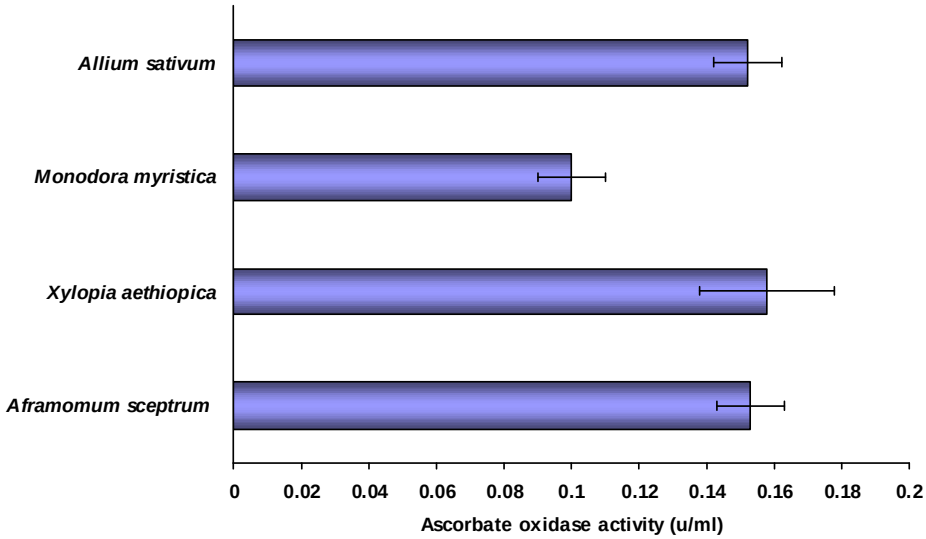

Figure 2. Bar chart showing the distribution of ascorbate oxidase activity (u/ml) of the investigated spices.

Table 2. Reducing Power Assessment (%) of different concentrations of water, ethanol methanol and heated water extracts of *Aframomum sceptrum, Xylopia aethiopica, Monodora myristica and Allium sativum.*

Plants	Water extract			Ethanol extract		
	A	B	C	A	B	C
Aframomum sceptrum	6.22±0.21	19.21±0.32	78060±0.53	178.15±1.57	315.58 ±0.53	472.51±1.74
Xylopia aethiopica	17.52±0.56	125.21±1.05	352.18±0.36	5.95±0.04	17.90 ±0.10	52.32±0.23
Monodora myristica	19.21±0.01	65.57±0.01	105.48±1.98	105.29±0.02	466. 90±0.01	677.19±1.03
Allium sativum	109.17±0.88	421.34±0.75	464.79±3.35	21.43±0.68	178 .75±1.08	331.64±1.28

A = 0.01 g/ml, B = 0.02 g/ml and C = 0.03 g/ml.

Table 2. Contd.

Plants	Methanol extract			Heated water extract		
	A	B	C	A	B	C
Aframomum sceptrum	118.52±0.02	390.58±4.84	681.27±0.33	47.01±0.01	72.13±0.11	99.23±0.20
Xylopia aethiopica	74.83±1.01	183.29±0.25	333.78±0.01	25.85±0.04	89.40±0.01	135.06±0.06
Monodora myristica	65.52±0.50	232.47±0.02	592.05±0.01	5.97±0.01	21.86±0.02	90.07±0.01
Allium sativum	60.44±0.55	121.82±1.00	287.14±1.02	166.79±3.72	343.63±3.02	651.87±1.40

comparable at P>0.05, but significantly different from that of *A. sativum.*

Reducing power

Table 2 indicated that the reducing powers of the various spice extracts were concentration dependent. The reducing ability increased with increasing concentrations. Results of the reducing power of the methanolic extracts of *A. sceptrum* and *X. aethiopica* were higher than that obtained from the three other extracting solvents (water, ethanol and heated water). The methanolic extract of *M. myristica* showed its highest reducing capacity. The table also revealed that the reducing power assessment of water and heated water of garlic (*A. sceptrum*) extract were higher than that of ethanol and methanol.

DISCUSSION

The use of naturally occurring antioxidants in the

preservation of food substances has been promoted because of concerns regarding the safety of synthetic antioxidants. Antioxidant may be defined as any substance that when present at low concentrations compared with those of the oxidizable substrate, significantly delays or inhibits oxidation of that substrate (Antolovich et al., 2002). The utilization of spices as food additives has been an age long practice carried out by different people in many parts of the world (Ifesan et al., 2006; Giese, 1994).

In this study, the phenolic content of all the spices investigated (*A. sceptrum, X. aethiopica, M. myristica* and *A. sativum*) were maximum in methanolic extract except in the *A. sativum*. This result is in agreement with those of Nurul et al. (2008) who also reported higher phenolic content in the mathanolic extracts in all aerial parts of *Barringtonia racemosa* than ethanolic or boiling water extract. Methanol is more polar than ethanol, and most polar solvent results in a greater yield extract of natural antioxidant compounds because most of them are polar compounds such as flavonoids (water is the most polar among the tested solvents) (Nurul et al., 2008; Chang et al., 1997). Phenolic compounds are important due to their ability to serve as antioxidants which are widely found in secondary products of medicinal plants (Wang et al., 2008). The antioxidant activity of phenolics is mainly due to their redox properties which play a role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Rice-Evans et al., 1997; Osawa, 1994).The ethanolic extract of *A. sceptrum* show high phenolic content (6.60±0.20 mg/g in terms of GAE). Water and heated water extracts of garlic (*A. sativum*) recorded high phenolic content when compared to the ethanolic or methanolic extract. This may be as a result that most of the phenolic compounds present in garlic are polar in nature.

Antioxidants are vital substances which possess the ability to protect the body from damage by free radical-induced oxidative stress (Souri et al., 2004). The total antioxidant capacity level of the heated water extract in all the spices studied was significantly higher than the level obtained for all other extractive solvents used. This result could suggest that the hydrophilic antioxidant compounds present in the spices are more. The result could also explain the popular use of these spices locally as hot soups for various disease conditions.

Foyer (1993) reported that ascorbate function as a reductant for many free radicals and it has been found in the chloroplast, cytosol, vacuole and extracellular compartments of the plant cells. In this study, ascorbate oxidase activities were comparable in three spices (*A. sceptrum, X. aethiopica and A. sceptrum*).

The reducing power assessment in this study increased with increasing concentrations. All the spices showed same pattern of increase in reductive potential. Kumaran and Karunakaran (2007) reported that the reductive properties are generally associated with the presence of reductones that exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. A similar result has been documented by Nurul et al. (2008).

Conclusion

It could be concluded from this study that the local spices investigated possessed antioxidant properties and can be by food and pharmaceutical industries as new potential sources of natural antioxidants. For further studies, the antioxidant properties of these spices should be screened along side with known antioxidants such as vitamin E, butylated hydroxylanisol (BHA) and butylated hydroxltoulene (BHT).

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