



Antioxidant properties of Ethiopian pepper in scavenging 1, 1-Diphenyl Picryl-2-hydrazyl radical and in suppressing rancidity in African breadfruit during storage

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Abstract

Fruit oil (N-hexane extract), ethanol and water extract of fruits, seeds and oil-extracted fruit residue of Ethiopian pepper spice were screened for radical scavenging activity, using DPPH radical. African breadfruit seed dhal and flour were preserved with the spice oil at 0, 0.5, 1.0, 1.5, 2.0 ml oil / 100g in black polyethylene bags at 26±2°C for 4 months. Ethanol and water extracts of fruits, seeds and oil-extracted fruit residue scavenged DPPH proportionally with increasing extract concentrations. The spice oil suppressed rancidity in stored African breadfruit seed dhal and flour in dose-dependent order. Scavenging activity increased with extracts concentrations. Ethanol extracts of seeds and whole fruits had higher slopes (0.996, 0.276) indicating better antioxidants than water extracts of seeds, whole fruits and oil-extracted fruit residue with lower slopes (0.311, 0.096, and 0.683). Seeds were better antioxidant than whole fruit or oil-extracted fruit residue. Ethiopian pepper fruits possess good antioxidant property.

Keywords: antioxidant property, Ethiopian pepper, DPPH

1. Introduction

African bread fruit (*Treculia africana*) is an unconventional food grain native to tropical region. The grain is a good source of protein (Iwe and Ngoddy, 2001; Giami *et al.*, 2004) ^[11, 8] readily available, though not relatively cheap but easily become rancid and deteriorate on storage due to autoxidation of the lipid component (Adindu and Williams, 2003) ^[1]. This reduces its food potential and availability in the market, and may pose serious setback in its utilization in the Nigerian food industries and beyond. Antioxidants are chemicals which delay the start or slow the rate of lipid oxidation in biological systems, including food materials such as African bread fruit seeds. They inhibit the formation of free alkyl radicals at the initiation stage or intercept the propagation of the free radical chains. They inhibit oxidative reaction of lipids in biological systems either by donating hydrogen to free radicals to form stable compounds or by formation of a complex between the antioxidant radical rings and lipid radicals. They require very low activation energy to abstract and donate hydrogen to free radicals to form stable complexes; and when the hydrogen is donated, the resulting antioxidant free radical ring cannot initiate another free radical formation since they are stabilized (Falowo *et al.*, 2014) ^[6]. Antioxidants can be of synthetic or of natural origin. Common synthetic antioxidants in use include butylated hydroxyl anisol (BHA), Butylated hydroxyl toluene (BHT), pyrogallol acid (PG) and tertiary butyl hydroxyl quinone (TBHQ). The natural antioxidants in commercial application include tocopherol, nordihydrogurtic acid (NDGA), sesamol and gossypol. Recently, natural antioxidants are preferred to synthetic antioxidants by consumers (Manach *et al.*, 2004; Amenteros *et al.*, 2013) ^[13, 2]. Most synthetic antioxidants are seriously

suspected to pose health hazards to warm blooded animals, constitute risks of environmental contamination and degrade to obnoxious residues when used (Fang *et al.*, 2002) ^[7]. In addition to imparting distinctive flavours, spices contain antioxidant properties and inhibit rancid flavour development associated with lipid oxidation (Okwu *et al.*, 2003; Schnella *et al.*, 2010; Radha *et al.*, 2014) ^[15, 21, 17]. Spices such as clove, cinnamon, turmeric, black pepper, ginger, garlic and onion have been shown to exhibit antioxidant properties in different food systems (Vasavada *et al.*, 2006; Vasavada *et al.*, 2006; Falowo *et al.*, 2014) ^[5, 5, 6]. Spices have antioxidant properties due to the presence of some phytochemical compounds such as flavonoids, terpenoids, lignans and polyphenolics (Shahidi and Nacz, 2004) ^[22, 23]. However, their use may be limited in some foods due to their characteristic astringency, aroma and flavour. Lipid oxidation is the major cause of food deterioration, and may decrease the nutritional value of such food by forming potentially toxic products during cooking, processing and storage (Shahidi and Nacz, 2004) ^[22-23]. Oxidation is a normal metabolic process that occurs in living cells, leading to the production of oxygen-derived radicals known as reactive oxygen species (ROS) (Xu *et al.*, 2007; Ravipati *et al.*, 2012.) ^[29-30, 18]. Oxidants are by-products of normal metabolism, but if not controlled, may cause many chronic and degenerative diseases in human (Fang *et al.*, 2002; Hayes, 2005) ^[7, 9]. There is the need to inhibit lipid oxidation of African bread fruit using indigenous spices such as Ethiopian pepper (*Xylopi aethiopia*) during storage to fully exploit its food potentials. This study was therefore aimed at investigating the radical scavenging activity of Ethiopian pepper and its antioxidant ability to inhibit lipid oxidation in stored African bread fruit seed dhal and flour.

2. Materials and methods

2.1 Materials

Dry wholesome undehulled African breadfruit (*Treculia africana* Decne) seeds were purchased from peasant farmers at Ogbede-Aku market of Igbo-Etiti Local Government Area (LGA), while Ethiopian pepper (*Xylopiya aethiopiya* Dunal A.Rich) fruits were obtained from commercial stockers at the Ogige Main Market, Nsukka, both in Enugu State.

2.2 Extraction of spice oil of Ethiopian pepper (*Xylopiya aethiopiya*)

Ethiopian pepper spices were extracted using maceration technique. The fruits were sun-dried (26 ± 2 °C) for 18 hours within three days and then equilibrated at 50°C in hot air oven for 24 hours. The fruits (400 g) were then milled into flour using an attrition mill (Perten 310, Perten Instruments, Sweden). The flour was divided into two equal parts. One part (200 g) was stored in an air-tight vial and was used for proximate analysis while the other part (200 g) was mixed with 500 ml of n-hexane. This was mixed for 30 minutes on a rotary shaker and then rested for 48 hours. The supernatant was then decanted and the solvent (n-hexane) evaporated on a water bath maintained at 60°C for 30 minutes. The resulting fruit oil was stored in Bijou bottle until used for the analysis.

2.3 Preparation of aqueous and ethanol extracts of spice

Ethiopian pepper (*Xylopiya aethiopiya*) fruits (20 g) and fruit residues after oil extraction were each cleaned and separately milled into flour using a hand-operated kitchen (colloid) mill. Ten grams (10 g) of each flour was separately mixed and extracted with 200 ml of ethanol or distilled water for 10 minutes before resting for 24 hours. Extraction was repeated for another 20 minutes before filtering through a double fold Whatman no 5 filter paper. Extracts were stoppered in the holding tube for immediate use.

2.4 Cleaning and processing of African breadfruit (*Treculia africana* Decne) seeds

African bread fruit seeds (1 kg) were parboiled in excess boiling water for 15 minutes, drained and cracked in a hand-operated (kitchen) colloid mill to remove the hulls from seeds. The dehulled seeds (dhal) were oven-dried at 50°C for 48 hours, and half of the dried dhal was milled into flour. The dhal and flour were used for antioxidant study. Five duplicated samples (100 g each) of the seed dhal and flour were separately mixed with different concentrations (0, 0.5, 1.0, 1.5, 2.0 ml) of *Xylopiya aethiopiya* oil, using a kitchen mixer (Kenwood). Each sample was bagged in nylon sack and stored at ambient temperature (26 ± 2 °C) for 4 months for oxidation study.

2.5 Determination of radical scavenging activity

Radical scavenging analysis was determined using the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Ethanol extracts (1000 µl each) of Ethiopian pepper fruit with different concentrations of extract (1.0 µl to 200 µl) were mixed with approximately 0.004 µl of DPPH solution containing 4 mg of DPPH in 100 mg of ethanol in 15-ml test tubes, and the volumes made up to 1200 µl with ethanol (Roberta *et al.*, 2006; Xu and Chang, 2007) [19, 29-30]. The tubes were incubated for 30 minutes at room temperature

(26 ± 2 °C) in the dark. Absolute ethanol was used as blank. The absorbance of the DPPH solution and resulting mixtures were measured at 517 nm with spectrophotometer and converted to antioxidant activity (AA%) as percentage inhibition relative to control.

2.6 Thiobarbituric acid (TBA) test in stored *Treculia africana* seed dhal and flour

Thiobarbituric acid (TBA) reactive substances (TBARS) were determined as described by Buege and Aust (1978) [3]. Five grams of each the stored dhal or flour was mixed with 2.5 ml of the stock solution containing 0.375% TBA (Sigma chemical Co., St. Louis Mo, U.S.A.), 15% Trichloro-acetic acide (TCA) (Mallinkrodt Beker Inc., Paris ky, USA) and 0.25 N HCL. The mixtures were heated for 10 minutes in a boiling water bath (100 °C) to develop a pink colour, cooled in tap water and centrifuged (Beckman coulter Ltd Palo, Alto, Calitonia, U.S.A) at 3000 rpm for 20 minutes. The absorbance of the supernatants was measured spectrophotometrically (Spectronic 21d, Multon Roy, Rochester Ny, U.S.A) at 532 nm against a blank that contained all the reagents except the *Treculia* seed or flour. The absorbance values were multiplied by a factor of 7.8 to obtain the TBARS values (*Van der Sluis et al.*, 2000) [26].

2.7 Data analysis

All data were analysed with SAS computer software (SAS, 2002 – 2003). The analysis of variance (ANOVA) mixed procedure (mixed) and Fisher's least significant difference (LSD) was used to ascertain significant effects at $P < 0.05$ level of significance among treatments.

3. Results and Discussions

3.1 Free radical scavenging activity of the spice extracts against 1,1-Diphenyl -2- picryl hydrazyl (DPPH)

The radical DPPH has been widely used in the model systems to investigate the scavenging activities of several natural compounds like phenolics, anthocyanins and crude extracts of plants (Huang *et al.*, 2005) [10]. The radical DPPH was first used because it is very sensitive, simple and rapid; and was therefore very convenient for screening many samples of different polarity (Koleva *et al.*, 2002) [12]. Antioxidants scavenge DPPH radical through the donation of a proton, thereby forming the reduced DPPH, 2,2-Dihydrazyl-1-picryl hydrazine. The colour of the medium changes from purple to yellow after reduction; and this can be quantified by its decrease of absorbance at wavelength of 517 nm. Radical scavenging activity increases with increasing percentage of the free radical inhibition. The free radical scavenging of aqueous and ethanolic extracts of various components of the spices were tested. The Figures 1, 2 and 3 showed the radical scavenging activities of water extract of whole fruit, water and ethanol extracts of seed, and water and ethanol extracts of the fruit pods of *Xylopiya aethiopiya*, respectively. The degree of discolouration indicates the scavenging potential and activity of antioxidant extracts. All the different water and ethanolic extracts of the *Xylopiya* fruit components screened had appreciably high scavenging activity. The regression analysis of the scavenging activity of *Xylopiya* fruit parts showed a high linear dependence of scavenging activity on the concentrations of the spice extracts (Figures 1, 2, and 3).

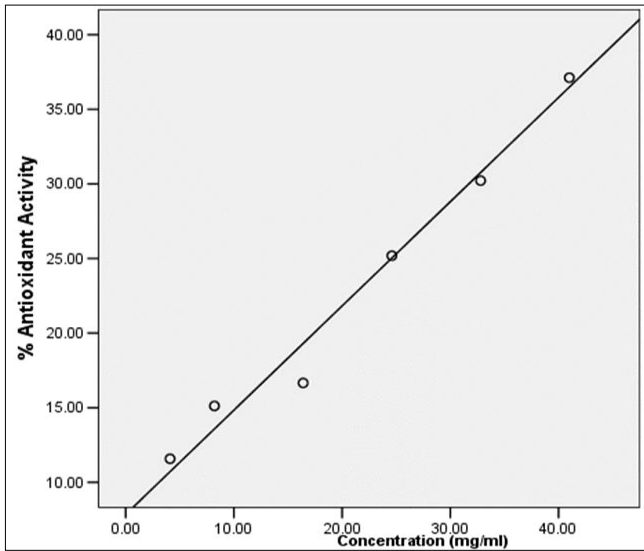


Fig 1: DPPH radical scavenging activity of water extract from *Xylopiya aethiopiya* whole fruit residue after oil extraction of the spice extract.

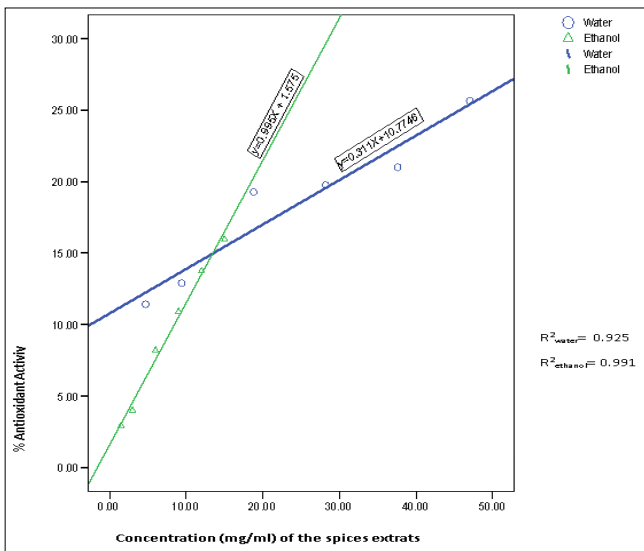


Fig 2: DPPH radical scavenging activity of extracts from *Xylopiya aethiopiya* seeds.

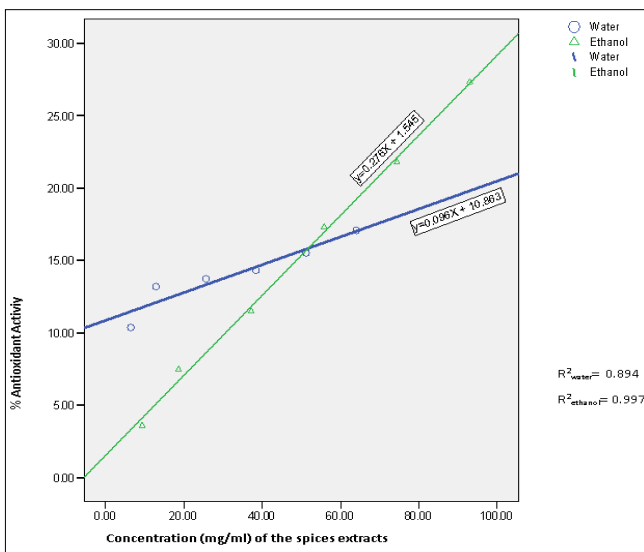


Fig 3: DPPH radical scavenging activity of ethanol and water extracts from *Xylopiya aethiopiya* fruits from *Xylopiya aethiopiya* fruit pods.

3.2 Comparison of TBA values of Treculia seed treated with Xylopiya aethiopiya spice oil

Main effect of treatment (*Xylopiya* spice oil), spice oil level (0%, 0.5%, 1.0%, 1.5%, 2.0%) and storage time (3, 5, 10, 17, 25, 31 days) significantly ($p < 0.05$) affected the mean TBA values of dehulled *Treculia* seed dhal and flour (Table 1). The 2-way interaction of spice level x day and the 3-way interaction of treatment x spice oil level x days of storage also significantly affected the mean TBA values of the samples ($p < 0.05$). Mean TBA values for the 3-way interaction for the seed dhal are shown in Figure 4. Mean TBA values on the 3rd day of storage ranged from 0.18 for SX2.0 (2% oil) to 0.57 for SX.5 (0.5% oil); and ranged from 0.93 for SX2.0 to 1.46 for SX.5 on the 31st day of the storage period. After 10 days of storage, mean TBA values for SX00 and SX2.0 did not differ significantly ($p > 0.05$) but were significantly higher ($p < 0.05$) than those of SX1.0 (1% oil), SX1.5 (1.5% oil) and SX4 (2% oil level). Mean TBA values increased with increased storage days but decreased significantly ($p < 0.05$) with increased spice oil concentrations. Thus *Xylopiya aethiopiya* spice oil suppressed rancidity development in *Treculia africana* seed dhal. Figure 5 showed the mean TBA values of *Treculia* seed flour treated with different concentrations of *Xylopiya* essential oil (0%, 0.5%, 1.0%, 1.5% and 2.0%) stored at 26 ± 2 °C for 25 days. The mean TBA values were observed to increase linearly with storage days but were suppressed significantly ($p < 0.05$) with increasing *Xylopiya* oil concentration in the samples Thus mean TBA values ranged from 0.41 and 0.21 on the 3rd day to 1.37 and 0.94 on the 25th day for FX00 (0% oil level and FX2.0 0% oil level), respectively. The meant TBA values for the 3-way interaction for 4 months storage of the *Treculia* seed dhal and flour with the various levels of *Xylopiya* spice oil treatment are shown in Table 2. Increasing spice oil concentration decreased the mean TBA values significantly ($p < 0.05$) in both African breadfruits dhal and flour, but the mean TBA values increased in that order with the storage time (months). On the 4th month of storage, SX00 (0% oil) and FX00 (0% oil) had mean TBA values of 1.61 and 1.74 while SX2.0 (2.0% oil) and FX2.0 (2.0% oil) had mean TBA values of 0.93 and 1.06 respectively. Thus, *Xylopiya aethiopiya* oil significantly ($p < 0.05$) suppressed rancidity development in both the seed dhal and flour. It has antioxidant potency just like extracts and oils of many other common spices such as rosemary and sage (Chang *et al.*, 1997; Ozkan *et al.*, 2002) [4, 16], black pepper (Okwu, 2003) [15] and several other spices of international trade (Dwivedi *et al.*, 2006; Zahin *et al.*, 2009; Zhang *et al.*, 2011) [5, 31, 32].

Table 1: Summary of significance ($p < 0.05$) for African breadfruit seed dhal and flour treated with *Xylopiya aethiopiya* oil, stored at 26 ± 2 °c stored for 31 days.

		Df	TBA	P level
Seed dhal	Treatment	1	*	0.0001*
	Spice oil conc.	4	*	0.0001*
	Day of storage	5	*	0.0001*
	Spice oil conc. x day	20	*	0.0001*
	Treatment x spice oil conc. x day	29	*	0.0001*
Seed flour	Treatment	1	*	0.0001*
	Spice oil conc.	4	*	0.0001*
	Day of storage	4	*	0.0001*
	Spice oil conc. x day	16	*	0.0001*
	Treatment x spice oil conc. x day	24	*	0.0001*

Table 2: Changes in TBA value (n=4) of *Treculia africana* seed dhal and flour treated with *Xylopiya aethiopiaca* oil

Sample code oil conc.			Storage Time (Months)				
			0	1	2	3	4
Seed	SX00	0.00	0.89±0.03 ^b	1.35±0.00 ^a	1.43±0.00 ^c	1.46±0.00 ^d	1.61±0.00 ^a
	SX.5	0.50	0.79±0.00 ^b	0.89±0.00 ^a	1.03±0.00 ^b	1.32±0.00 ^d	1.62±0.00 ^c
	SX1.0	1.00	0.67±0.00 ^b	1.24±0.00 ^c	1.29±0.00 ^c	1.40±0.00 ^c	1.54±0.00 ^d
	SX1.5	1.50	0.68±0.00 ^b	1.00±0.00 ^b	1.06±0.00 ^d	1.10±0.00 ^f	1.02±0.00 ^j
	SX2.0	2.00	0.68±0.24 ^b	0.89±0.00 ^b	0.92±0.00 ^g	0.93±0.00 ^h	0.93±0.00 ^g
Flour	FX00	0.00	1.16 ±0.14 ^a	1.32±0.00 ^c	1.47±0.00 ^a	1.73±0.00 ^a	1.74±0.00 ^a
	FX.5	0.50	1.18±0.00 ^a	1.24±0.00 ^b	1.27±0.00 ^{cd}	1.10±0.00 ^e	1.19±0.00 ^e
	FX1.0	1.00	1.16±0.00 ^a	1.21±0.00 ^d	1.24±0.00 ^a	1.10±0.00 ^f	1.15±0.00 ^f
	FX1.5	1.50	1.15±0.00 ^a	0.92±0.00 ^f	0.98±0.00 ^f	1.06±0.00 ^f	1.13±0.00 ^g
	FX2.0	2.00	0.61±0.00 ^b	0.91±0.00 ^f	0.95±0.00 ^{de}	1.04±0.00 ^g	1.05±0.00 ^h
L S D			0.59	0.03	0.08	0.03	0.02

Data values are expressed as mean ± standard deviation (n=4) values within each month in the same column with the same superscript are not significantly different (p>0.05). SX00 to SX2.0 and FX00 to FX2.0 are 100g samples of African bread fruit seed dhal (SX) and flour (FX) treated with 0.00, 0.5, 1.0, 1.5 and 2.0% v/w *Xylopiya aethiopiaca* oil concentrations respectively.

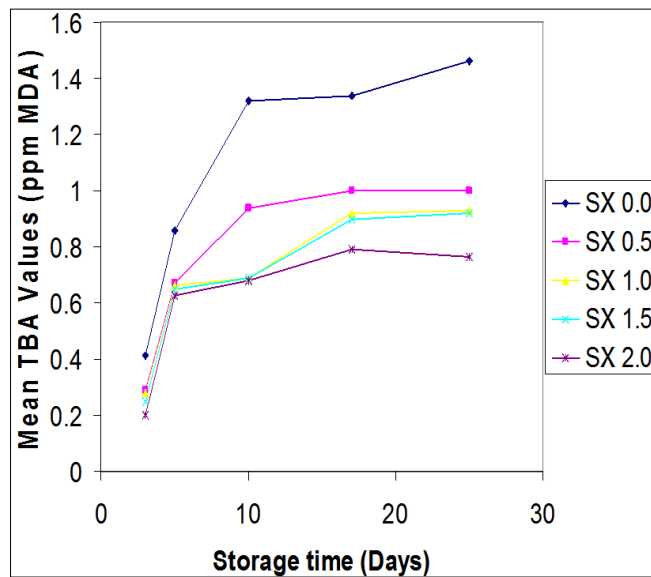


Fig 4: Effect of *Xylopiya aethiopiaca* spice oil concentration (0.0, 0.5%, 1.0%, 1.5%, 2.0% w/w basis) on thiobarbituric acid (TBA) value of *Treculia africana* during storage at 26 ±2°C.

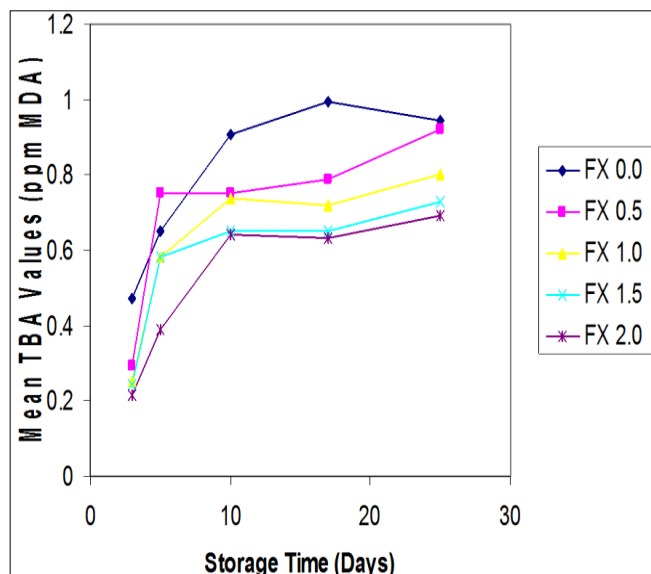


Fig 5: Effect of *Xylopiya aethiopiaca* spice oil concentration (0.0, 0.5%, 1.0%, 1.5%, 2.0% w/w basis) on thiobarbituric acid (TBA) value of *Treculia africana* seed flour during storage at 26 ±2°C.

4. Conclusion

This study showed that Ethiopian pepper (*Xylopiya aethiopiaca*) exhibited antioxidant properties in scavenging DPPH radical and in inhibiting rancidity development in African breadfruit (*Treculia africana*) seed dhal and flour during storage. The aqueous and ethanol extracts of ground fruits, seeds and oil-extracted fruit residue scavenged DPPH radical in concentration-dependent order and oil extract suppressed malonaldehyde formation (TBA values) in African breadfruit in dose-dependent order. Ground Ethiopian pepper fruit, ethanol- and water-extracts or its oil can be used to suppress lipid oxidation in model food systems.

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6. Conflict of interest: None.

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