EVALUATION OF NUTRITIVE POTENTIAL AND ANTI-OXIDATIVE PROPERTIES OF AFRICAN NUTMEG (*MONODORA MYRISTICA)*

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ABSTRACT
Monodora myristica seed was evaluated for its nutritional and preservative potentials. The seed was assessed for its proximate composition, and its anti-oxidative activities, which include its peroxide value development, chelating ability, scavenging ability and reduction power. The results showed that the seed contained 13.87% crude protein, 38.13% ether extract, 5.66% ash, 5.02% crude fibre and 35.74% nitrogen-free extract on dry matter basis. The peroxide values of different concentrations of the seed extract on soyabean oil were determined for 4 weeks. The values ranged from 8.30 to 22.87mEq/Kg for the control (0 ppm), 8.30 to 22.80mEq/Kg for 2000 ppm, 8.30 to 21.27mEq/Kg for 4000 ppm, 8.30 to 20.53 mEq/Kg for 6000 ppm and 8.30 to 19.47mEq/Kg for 8000 ppm within the 4-week period. The scavenging values ranged from 39.80 to 87.80%, chelating values ranged from 57.13 to 93.01% while the reduction values ranged from 0.885 to 2.672 units at the different concentrations (C1 = 2000 ppm, C2 = 4000 ppm, C3 = 6000 ppm, and C4 = 8000 ppm). Result of the analysis of *Monodora myristica* showed it possessed strong anti-oxidant effects and as such can be used as a feed preservative.

Keywords: African Nutmeg, Antioxidative activity and Nutritive potential.

INTRODUCTION
Fats and oils undergo changes during storage and these changes result in production of an unpleasant taste and odour commonly referred to as rancidity (Onwuka, 2005). Oxidation is a major factor for quality deterioration (rancidity) of edible fats and oils. Antioxidants are often added to fat-containing foods in order to delay the onset or slow the development of rancidity due to oxidation (Salah *et al.*, 1995; Del-Rio *et al.*, 1997; Okwu, 2004; Wikipedia, 2010). Natural antioxidants incude flavonoids, polyphenols, ascorbic acid (Vitamin C) and tocopherols (Vitamin E). Synthetic antioxidants presently in use as feed additives include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), among others (Okwu, 2004; Wikipedia, 2010). However, in recent times there has been heightened advocacy for organic agriculture (which implies zero or minimal use of synthetic inputs in all fields of agriculture) coupled with an increased questioning of the use of synthetic additives and antioxidants especially antibiotics by sensitive consumers (Adegoke *et al.*, 1998). The result of these expressed concerns is the current restriction and even ban in the use of some synthetic feed additives in livestock rations in some countries. This has brought with it an increase in the use of plant products as alternatives. Such plant products include spices.

Spices are a class of pungent or aromatic substances of vegetable origin such as pepper, onion, garlic, ginger, nutmeg, among others (Webster, 1989). They are pronutrients used in relatively small amounts to improve the intrinsic value of the nutrient mix in animal diets. They have been reported to show anti-inflammatory (Reddy and Lokesh, 1994), antidiabetic (Broadhurst *et al.*, 2000), antioxidative (Nakatani, 2000; Krause and Ternes, 2000), and anti-carcinogenic (Surh, 2002) activities, as well as to increase feed intake (Wenk, 2003). Spice extracts can provide inhibition of oxidative rancidity and retard the development of "warmed-over" flavour in some products (Peter, 1997).

African nutmeg (*Monodora myristica*) is a spice and an edible plant belonging to the *Annonaceae* family. In vernacular it is called "Ehuru" by the Igbos, "Abo Lakoshe" in Yoruba and "Ebenoyoba" in Edo. The seeds

are aromatic and sold all over West Africa as Spice. Bede and Chigbu (2007) reported that "Ehuru" can be used to retard oxidation and hence extend the freshness of palm oil.

Inspite of all this potential benefits the use of spices and *Monodora myristica* in particular as antioxidants in feed preservation is not popular and not much information exists on this. It is thus the aim of this research to evaluate the antioxidative properties and chemically investigate the nutritive potential of African nutmeg *(Monodora myristica*).

MATERIALS AND METHODS

African nutmeg seeds were sourced from Umuahia Main Market in Abia State of Nigeria. The seeds from fresh pulp were collected and cleaned to remove all foreign matter and damaged seeds. It was then sun dried and maintained at room temperature. The seeds were then separated from the coat and ground into a powdery form. Thereafter chemical analyses and anti-oxidation analysis were carried out.

Chemical analyses

Determination of proximate composition of the sample was by A.O.A.C. (1990) procedure, employing the micro-Kjeldahl method for crude protein and the Soxhlet extraction method for ether extract. Nitrogen-free extract value was obtained by difference.

a) Determination of chelating ability, scavenging ability, and reduction power of M. myristica extract

A 20g sample of the dried African nutmeg powder was mixed in 70% methanol (1,000mL) and kept in the shaking incubator at 25^0C for 3 days and filtered in vacuum using Whatman No. 1 filter paper. Later, solvent fractionation of 70% methanol extract was separately done with n-hexane, chloroform and ethyl acetate. After solvent fractionation, both aqueous and organic fractions were evaluated for antioxidant activities, which included chelating ability, scavenging ability, and reduction power as well as assessing the peroxide value development.

The method of Decker and Welch (1990) was used for determination of ferrous ion chelating ability. The Fe^{2+} was monitored by the measurement of the Fe^{2+}-Ferrozine complex.
10mL of the African nutmeg extract containing 0.05 - 10mg/mL was dispersed into a beaker. 1mL of 0.002m Fe^{2+} solution was added and followed by 2mL of 0.05m Ferrozine solution. The mixture was shaken to mix well and then allowed to stand for 10 mins at room temperature. The absorbance was measured in Jenway spectrophotometer at a wave length of 562nm. A control was set up but without the seed extract. It was treated as discussed above. Absorbance was also measured at 562 nm. The percentage ion chelating effect was calculated using the formula below:

$$\% \text{ CE} = \frac{(1-Au)}{As} \times \frac{100}{1}$$

Where: CE = chelating effect
Au = Absorbance of the test sample
As = Absorbance of control

The scavenging effect was determined using the methods described by Nagai *et al.* (2001), Chung *et al.* (1997) and Muller (1995). Extract of African nutmeg sample was made with 50% ethanol. An aliquot of the extract diluents (0.5 to 10mg) as well as antioxidant - Butylated hydroxyl toluene (BHT) were used in the same test. The test extract was mixed with equal volume of 0.01m Tris-HCL buffer (pH 7.4) and the 1ml of 0.05m 1,1-diphenyl 1-2- picryl hydrazl (DPPH) in ethanol. The mixture was shaken vigorously and left for 20 mins in a dark cupboard at room temperature. The absorbance of the resulting solution was measured at 517 nm in a spectrophotometer with the reagent blank at zero. A control without the extract was run as well. The percentage scavenging effect was calculated using the relationship below.

$$\%SE = \frac{(1\text{-absorbance of sample at 517nm})}{\text{Absorbance of control at 517nm}} \times \frac{100}{1}$$

Reducing power of African nutmeg extract was determined using the method reported by Onyaizu (1986). A 2.5mL fraction of African nutmeg extract was mixed with 2.5mL of phosphate buffer solution (200mM, pH 6.6) and 2.5mL of 1% potassium ferricyanide. The mixture was placed in a water bath for 20 minutes at 50^{o}C. The resulting solution was cooled rapidly, mixed with 2.5mL of 10% trichloroacetic acid and centrifuged at 3000rpm for 10min. A 5.0 mL fraction from the supernatant was mixed with 5.0 mL of distilled water and 1 mL of 1% $FeCl_3$. Absorbance of the resultant mixture was measured at 700nm after 10min. The higher the absorbance the stronger the reducing power.The test was carried out alongside standard antioxidants - Butylated hydrotoluene (BHT) approximately 0.02 to 0.62 mg/ml. All tests were conducted in triplicates.

b) Evaluating effect of M. myristica crude extract on lipid oxidation

The African nutmeg extract obtained was used on refined Soya bean oil at different levels to determine the peroxide values. The method described by Pearson (1991) was adopted. The range of levels used were 2,000, 4,000, 6,000 and 8,000 ppm of the crude extract. 10ml of Soya bean oil was used for each level of the crude extract. The samples were left for a period of 4 weeks. The peroxide values were measured at intervals of 7 days for the 4-weeks period. Thus, the readings were taken at the 7th, 14th, 21st and 28th days. To measure the peroxide value, 1g of the sample was weighed out into a clean dry boiling tube and while still liquid, 1g of powdered potassium iodide and 20ml of solvent mixture (2 vol glacial acetic acid + 1 vol chloroform) were added. The tube was placed in boiling water so that the liquid boils within 30 seconds. The content of the tube was poured quickly into a flask containing 20ml of potassium iodide solution (5%) and the tube washed out twice with 25ml water and titrated with 0.002M sodium triosulphate solution using starch.

A blank was run at the same time. The antioxidant effectiveness was calculated as follows:

$$AE = \frac{\text{Peroxide value} - \text{PV of test}}{\text{PV of control}} \times \frac{100}{1}$$

The value was compared with Butylated hydroxyl toluene (BHT) as standard antioxidant.

RESULTS AND DISCUSSION

Result of the proximate composition of the test seed (*Monodora myristica*) is shown in Table 1. The values obtained were 13.85, 38.13, 5.66, 5.02 and 35.74% for crude protein, ether extract, ash, crude fibre and nirogen-free extract, respectively. The crude protein value is higher when compared with other known spices such as ginger (8.58%), onion (10.45%) and Ashanti pepper (12.50%) (Nwinuka *et al.*, 2005). The result shows that the seed is high in fat as revealed by the ether extract value. Oil is a very strong solvent for important vitamins like Vitamins A and E (Bede and Chigbu, 2007) with some antioxidative effects. The high oil content of the test seed may imply a rich source of fat soluble vitamins. The presence of these vitamins in any seed confers on such seed the potentials of having antioxidant activities. One of the ways of measuring the antioxidant activity of a test sample is its ability to retard or delay rancidity in foods or feed and also prevent flavor deterioration due to oxidation (Pearson, 1976).

Table 1: Proximate Composition of *Monodora myristica* seed

Constituents	Mean value (%)
Crude protein	13.85
Ether extract	38.13
Ash	5.66
Crude fibre	5.02
NFE	35.74

NFE= Nitrogen-free extract.

Table 2 shows changes in peroxide value of soyabean oil treated with different concentrations of the test seed extract. Onwuka (2005) reported that peroxide tests are used to monitor the development of rancidity through the evaluation of the quantity of peroxide generated in the product (initiation product of oxidation). From the table, it was observed that the peroxide value of the test oil without the extract measured 8.30mEq/Kg at day zero and rose to 22.87mEq/Kg at end of four weeks of storage. This represented an increase of 175.54%. The sample treated with the highest concentration of 8000 ppm (C_4) increased from 8.30 to 19.47mEq/Kg, an increase of 134.58%. Deterioration due to oxidation started in all the treatments. However, the oxidation did not reach actual rancidity level in any of the treatments until the 21st day. In the 8000 ppm treatment, rancidity was never reached even up till the 28th day. Onwuka (2005) reported that oil spoilage begins to occur at peroxide value of 20 – 40mEq/kg.

Table 2: Peroxide Values of Soya bean oil Treated with Monod*ora myristica* Seed Extract.

	Peroxide value (mEq/kg)				
Treatment	**0 day**	**7 day**	**14 day**	**21 day**	**28 day**
C_0	8.30	10.07	16.47	22.67	22.87
C_1	8.30	9.73	15.17	21.73	21.80
C_2	8.30	9.67	15.13	21.47	21.27
C_3	8.30	8.53	13.73	20.40	20.53
C_4	8.30	8.83	12.47	19.07	19.47

C_1 = Concentration at 2000 ppm **C_3 = Concentration at 6000 ppm**
C_2=Concentration at 4000 ppm **C_4 = Concentration at 8000 ppm**
C_o= Control (Test oil without *Monodora myristica* extract)

Table 3 shows the rate of increase of peroxide value of soyabean oil treated with *Monodora myristica* seed extract. The rate of increase of the peroxide value decreased as the concentration of *Monodora myristica* seed extract increased at week one, two and on the average basis, except at week three where there was no established trend. Also, there was increasing rate of peroxide value as time progressed up to the third week in all the treated samples, but in the control sample the increasing rate stopped at the second week and thereafter increased at decreasing rate.

Table 3: Rate of increase of Peroxide Value of Soya bean oil Treated with *Monodora myristica* Seed Extract.

Treatment	Peroxide Value (mEq/ kg/day)			
	7	14	21	28
C_0	0.25	0.91	0.89	0.68
C_1	0.20	0.85	0.87	0.64
C_2	0.20	0.78	0.91	0.63
C_3	0.03	0.74	0.95	0.58
C_4	0.08	0.52	0.94	0.53

C_1 = Concentration at 2000 ppm, C_2=Concentration at 4000 ppm, C_3 = Concentration at 6000 ppm, C_4 = Concentration at 8000 ppm, C_o= Control (Test oil without *Monodora myristica* extract)

The implication of the observed rates is that increasing of the seed extracts in a medium increases retardation of oxidation or rancidity in that medium. This means that higher concentrations of *Monodora myristica* seed extract have greater ability to check or retard rancidity of oil.Table 4 shows the relative scavenging, reducing and chelating effects of the test seed extract using Butylated hydroxyl toluene (BHT) as standard antioxidant at 100% performance as a standard for comparison.

At concentrations of 2000 - 8000 ppm ($C_1 – C_4$), the scavenging effect increased from 57.13 to 93.01% and chelating activity from 57.13% to 93.01%. Similarly, the reducing power increased from 0.885 to 2.672 units at the same concentrations. Scavenging, reducing and chelating effects are the three (3) factors that predict anti-oxidative activities. Also, the scavenging, reducing and chelating values obtained for C_4 (8000 ppm) were close to the values obtained for Butylated hydroxyl toluene (BHT), a standard antioxidant.

Table 4. Anti-oxidative Activities of *Monodora mysristica* Seed Extract

Sample	Scavenging	Reduction	Chelating
BHT	100	2.860	100
C_1	39.80	0.885	57.13
C_2	49.82	1.562	64.52
C_3	73.09	2.480	83.36
C_4	87.80	2.672	93.01

C1= Concentration at 2000 ppm, C_3= Concentration at 4000 ppm, C2 = Concentration at 6000 ppm C4= Concentration at 8000 ppm, BHT = Butylated hydroxyl toluene

The reduction activity implies that the test seed extract acts as an electron donor and can react with free radicals, converting them to more stable state (Miller, 2000). The scavenging and chelating effects of the test seed extract imply that the extract can protect the body cells of consumers from oxidative damage by mopping up and bonding with free radicals (Salah *et al.*, 1995; Okwu, 2004; Okwu and Josiah, 2006). The implication of these is that anti-oxidative activity of the *Monodora myristica* seed extract increases with its concentration in Soya bean oil.

As observed earlier, the test seed extract is rich in essential oil. This supposes that the anti-oxidative effect could be due to fat soluble vitamins (A, E) which are known to be strong anti-oxidants. Furthermore, the anti-oxidant effectiveness of the seed extract can also be due to phytochemicals present in the seed. Okwu (2004) observed that phytochemicals such as carotenoids, flavonoids, phenols, and vitamins possess strong anti-oxidative property.

CONCLUSION

From this study, it can be concluded that *Monodora myristica* has demonstrated anti-oxidant activities as shown in the high level of scavenging, reducing and chelating effects. Also, the protection of the vegetable oil from rancidity confirmed the same. However, it was noticed that the protection of oil from rancidity by the extract was effective at concentrations greater or equal to 8000 ppm. It is then recommended that the use of *Monodora myristica* as feed additive should be encouraged since it can protect oil rich feed from early rancidity. Also, consumers of the seed (man and animals) are most likely to enjoy protection against oxidative damage of the body cells. There is however, need for further studies on the seed with a view to finding any possible side effect at higher concentrations.

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