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Antioxidant Activities of Four Commonly Consumed Indigenous Spices of Nigeria as Affected by Extracting Solvents

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Abstract:- Four Nigerian spices, *Ocimum viride* (leaves), *Monodora myristica* (seeds), *Monodora tenuifolia* (seeds) and *Tetrepleura tetrapetra* (fruits) were screened for total phenol (TP) contents and antioxidant activities in five different extracting solvents using standard methods. Spice extracts scavenged 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, reduced iron (iii) chloride ($FeCl_3$), suppressed linoleic acid (LA) peroxidation in ferric thiocyanate (FTC) oxidizing systems and inhibited formation of thiobarbituric acid reactive substances (TBARS) in spice-treated beef and pork patties during storage (4°C, 14 days). Phenolic contents differed significantly ($p<0.05$) among the spices and extracting solvents. Methanol and methanol mixed solvents extracted higher TP contents than other solvents. Methanol (95%) extracts of *M. myristica, M. tenuifolia* and *O. viride,* and water extract of *T. tetrapetra* exhibited highest reducing power while acetone/water/acetic acid extracts exhibited highest radical scavenging capacity. Antioxidant properties of these spices increased with spice concentrations from high to low: *T. tetrapetra>O. viride>M. myristica>M. tenuifolia*.

Keywords:- *Antioxidant, Nigerian Spices, Extracting Solvents.*

I. INTRODUCTION

Oxidation involves the peroxidation of polyunsaturated fatty acids located in the membranes of living cells or in food systems [17]. Oxidation of lipids results in decreased colour, flavour and texture, nutritive and physiological values of lipids and lipid-soluble nutrients [9], [19]. Fat-soluble vitamins and essential fatty acids are destroyed [14]. Oxidants from oxidized foods cause pathological changes in the mucus membranes of alimentary tracts, inhibit enzyme activities, and increase cholesterol and peroxide contents in foods [14], [8]. In living cells, oxidation leads to production of energy necessary for essential cell activities. Oxygen derived free radicals, commonly known as reactive oxygen species (ROS) are also produced [25] as by-products of normal body metabolism. ROS if not controlled may cause oxidative stress on the organs. Oxidative stress is associated with the development of many chronic and degenerative diseases, including cancer, arteriosclerosis, neuronal degradation, hypertension and aging [9].

Antioxidants are substances which delay or inhibit oxidation and could be synthetic or natural. Natural antioxidants from plant materials, including cereals, legumes, fruits, vegetables, herbs and spices [4] are perceived by consumers to be better and safer than synthetic antioxidants [26]. Some conventional spices of International Trade that exhibit antioxidant properties include cinnamon, clove, onion, turmeric, black pepper, rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), garden thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) and majoram (*Origanum majoram*), and so many others [26], [1]. Such spices have been shown to possess phenols and phenolic acids, flavonoids, terpenes, terpenoids and lignans [18]. The antioxidant property in chili pepper is attributed partly to the presence of ascorbic acid, many flavonoids and phenolic acids [13].

Many other spices are still under investigation for antioxidant potency in food preservation and health promoting to consumers, following the increasing current demands for natural food and their health benefits [6]. Unlike with the exotic spices, information on phytochemical composition and antioxidant properties of many Nigerian spices is generally scarce. Phytochemical constituents of most Nigerian spices, including *Tetrapleura tetrapetra* (Hiohio; fruits), *Monodora myristica* (Ehuru; seeds), *Monodora tenuifolia* (Ehu; seeds) and *Ocimum viride* (Nchu-anwu; leaves) used in flavouring and preservation are not known. This study investigated phytochemical compositions and antioxidant properties of four commonly consumed Nigerian spices namely, *Tetrapleura tetrapetra, Monodora myristica, Monodora tenuifolia* and *Ocimum viride*. This would improve their application as preservative ingredients in food and as health–promoting ingredients to consumers.

II. MATERIALS AND METHODS

The spices *Tetrapleura tetrapetra (*Schum and Thonn*)* [12], *Monodora myristica* (Gaertn) [3], *Monodora tenuifolia (*Benth) and *Ocimum viride* (Willd) [12], were purchased from commercial stockers at Nsukka in Enugu State, Nigeria. Fresh beef and pork (thigh muscle) were purchased from meat sellers at the Ikpa market, Nsukka, Nigeria. The beef and pork were frozen (4°C) overnight (4.00 pm the first day to 8.50 am the next day) and thawed the next day by 8.50 am) before use. All the reagents used were of analytical grade.

A. Extracting solvents

Five solvent systems comprising distilled water, 95 % methanol, acetone / hexane (1:1,v/v), n-hexane / methanol / acetone (2:1:1, v/v/v) and acetone / water / acetic acid (70:29.5:0.5, v/v/v)were appropriately prepared based on the solvency of the constituent solvents and solubility characteristics of the extracting phytochemicals.

B. Preparation of spice extracts

The fruits of *Tetrapleura tetrapetra*, leaves of *Ocimum viride* and seeds of *Monodora myristica* and *Monodora tenuifolia* were sun-dried (72 hours), after which the *Monodora myristica* seeds were toasted (100 – 120 °C for 15 minutes) and cracked to recover nibs. Each of the spice was ground into powder using Hammer mill (Betsch 5657 GmbH, Germany). Two (2.0 g) grams of each spice was homogenized with 100 ml of each of the solvents for 3 minutes in sterile, tightly corked bottles. The mixtures were rested for 3 hours, re-homogenized for another 2 minutes and then filtered through cheese cloths into 100 ml bottles. Extracts were boiled (5 minutes) in water baths to inactivate inherent enzymes [7], cooled and filtered through Whatman No. 5 filter papers. Filtrates were concentrated to dryness in a rotary evaporator (70 °C) and tightly stored (4 °C) for use within 24 hours. Required concentrations [4, 8, 12, 16, 20 and 25 mg of dry matter/ml (D.M/mL)] for phytochemical and antioxidant analysis were reconstituted from the crude extracts using the extracting solvents.

C. Determination of total phenol contents

Total phenol content was determined using Folin-ciocalteau method [21]. Folin-ciocalteau method allows the estimation of all flavonoids, anthocyanins, and non-flavonoid phenolic compounds, including phenols and tannins, that is, all phenolics present in the sample [21]. The total phenol content of the various spices was determined by mixing 0.5 ml aliquot of freshly prepared sample extract with equal volume of water, 0.5 ml Folin-Ciocalteu's reagent, and 2.5 ml of saturated solution of sodium carbonate (Na_2C0_3). The absorbance was measured after 40 minutes at 725 nm. Garlic acid was used at concentrations of 0.0, 3.0, 6.0, 12.0, 18.0, 24.0 and 30.0 µg/ml to prepare total phenol standard curve. Total phenol content was extrapolated from the standard curve using the absorbance values and expressed as garlic acid equivalents (GAE/100g).

D. Measurement of reducing power of the crude extracts of spices.

Reducing power of the crude extracts of spices was determined according to the method of Yen and Chen (1995). The crude extracts (each of 0.2, 0.4, 0.6, 0.8 and 1.0 g of the spices extracted with 50 ml of the solvents) (5 ml) or BHT (5 ml) were separately mixed with equal volume of 0.2 M phosphate buffer (pH, 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes, after which an equal volume of 1 % trichloro acetic acid (TCA) was added to the mixture and then centrifuged at 3000 rpm for 10 minutes. The upper layer (the supernatant) of the suspension was mixed with distilled water and 0.1 % $FeCl_3$ in the ratio of 1:1:2 and the absorbance measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

E. Determination of antioxidant activity of crude extracts of the spices by the ferric Thiocyanate (CTC) method.

The ferric thiocyanate (FTC) method was adopted from [20]. The spice crude extracts (2.5 ml) were added to 2.5 ml of 95 % (V/V) ethanol, and then mixed with 4.1 ml of linoleic acid (2.51 % V/V) in 99.5 % (V/V) ethanol, 8 ml of 0.05 M phosphate buffer (PH 7.0), 3.9 ml of distilled water and then kept in the dark in screw- capped containers at 4°C. To 0.1 ml of this solution was added 9.7 ml of 75 % (V/V) ethanol and 0.1 ml of 30 % (W/V) ammonium thiocyanate. A 0.1 ml volume of 20 mM Ferrous chloride in 3.5 % (V/V) hydrochloric acid was added to the reaction mixture, and absorbance measured after 3 minutes at 500 nm repeatedly at interval of 24 hours until the control (no extract) reached maximum value.

$$(\%)\ \text{Inhibition} = 100 - \left\{ \frac{\text{Absorbance increase of sample} \quad X\ 100}{(\text{Absorbance increase of blank})} \right\}$$

F. Determination of free radical scavenging activity

Free radical scavenging activity of the spice extracts was determined using the radical 1, 1-diphenyl-2-Picrylhydrazyl (DPPH), which is widely used to evaluate the free radical scavenging activity of natural [2], [22]. A 1000 µL volume of the spice supernatant was mixed with 1000 µL of 0.4 M DPPH radical in ethanol solvent (0.004 % W/V). The mixture was left in the dark for 30 minutes before reading the absorbance at 517 nm. Radical scavenging was expressed as the inhibition percentage and was calculated using the formula of Yen and Duh (1994), where % Inhibition = $[(A_{DPPH} - A_{EXTRACT}) / A_{DPPH}] \times 100$, where A_{DPPH} = absorbance of DPPH radical at 517 nm and $A_{EXTRACT}$ = absorbance of extract of spice at 517 nm.

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III. RESULTS AND DISCUSSIONS

A. Effects of different extraction solvents on total phenol profiles of the spices

Table 1 reports total polyphenols contents in different solvent extracts of four Nigerian spices, namely dried leaves of *Ocimum viride*, dried fruits of *T. tetrapetra*, dried seeds of *Monodora myristica* and *Monodora tenuifolia*. The various solvent extraction systems [distilled water, 95% methanol, acetone/hexane (1:1; v/v), hexane/methanol/acetone (2:1:1; v/v/v), and acetone/water/acetic acid (70:29.5:0.5; v/v/v)] affected recovery of total phenols from the selected spices. Spice extracts from different extraction solvents differed significantly ($p < 0.05$) in their total phenol contents (TPCs). The TPCs of O. viride ranged from 8.77 to 12.33 garlic acid equivalent/100g; *M. myristica* from 2.43 to 7.45 GAE/100g; *M. tenuifolia* from 2.61 to 6.78 GAE/100g; and *T. tetrapetra* from 0.21 to 15.93 GAE/100g. The TPCs yields by the extracting solvents were in the following order from high to low: acetone/hexane > hexane/methanol/acetone > 95 % methanol > distilled water > acetone/water/acetic acid for *Ocimum viride*; distilled water > acetone/hexane > hexane/methanol/acetone > 95 % methanol > acetone water/acetic acid for *Monodora myristica;* acetone/hexane >distilled water > hexane/methanol/acetone > 95 % methanol > acetone/water/acetic acid for *Monodora tenuifolia;* and 95 % ethanol > acetone/hexane > hexane/methanol/acetone > distilled water > acetone/water/acetic acid for T*etrapleura tetrapetra.*

These results showed that the solvent combination acetone/water/acetic acid (70:29.5:0.5, v/v/v) was the best extractants for total phenol from any of the four spices. *Ocimum viride* had the highest total phenol content, followed by *Tetrapleura tetrapetra*. Generally, the four spices are good sources of phenols. Phenols are one of the major groups of non-nutritive dietary components that have been associated with the inhibition of cancer, atherosclerosis, as well as ameliorating age-related degenerative brain disorder [5], [30]. Phenolic phytochemicals inhibit autoxidation of unsaturated lipids, thus preventing formation of oxidized low-density lipoprotein (LDL) which has been associated with the incidence of cardiovascular diseases [9], [28]. Natural phenolic compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing 1st – chain initiation by scavenging initial radicals such as hydroxy radicals, binding metal ion catalyst, decomposing primary products of oxidation to nonradical species, and breaking chains to prevent continued hydrogen abstraction from substances [23]. Phenolic compounds play important roles in stabilizing lipid peroxidation and are associated with antioxidant activity [29]. According to Tanaka, Kwei, Nagashima, and Teguchi [24], polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in human when up to 1.0 mg is ingested daily from diets rich in fruits and vegetables.

B. Comparative effect of extracting solvents on reducing power of the four spices

Figure 1 compares effects of the five different extracting solvents on reducing power of the four spice extracts. Higher reducing power implies higher antioxidant activity. Antioxidant activities of extracts of each spice were significantly ($P<0.05$) influenced by the extracting solvents and the best extracting solvent for one spice was not always for another spice. Water extracts of *Ocimum viride.*

Species	Extracting solvents					
	ER	AW	AN	ONE	OL	MEAN
Ocimum viride	10.33^{b} ±0.03	16.77^{a}±0.04	7.68^{c} ±0.02	6.18^{e} ±0.01	9.03^{d} ±0.02	11.71±2.06
Monodora myristica	2.13^{e}±0.01	7.06^{a} ±0.02	4.82^{c} ±0.01	4.28^{d}± 0.02	6.18^{b} ± 0.02	5.66±1.12
Monodora tenuifolia	2.40^{d} ±0.00	8.85^{a} ± 0.01	2.63^{c} ± 0.02	2.30^{d}± 0.01	5.50^{b} ±0.02	5.04±1.81
Tetrapleura tetrapetra	7.17^{c} ±0.13	13.93^{a} ± 0.61	3.14^{d}±0.01	3.04^{d} ± 0.00	10.47^{b} ± 0.00	7.24±1.22

Table 1:- Effect of different extraction solvents on phenol profiles (GAE / 100 g) of spices

Data are means ± standard deviations (n = 3); values within each type of spice marked by the same letter within the same row are not significantly different ($p < 0.05$).ER = distilled water, OL = 95% methanol, ONE = acetone/hexane (1:1; v/v), AN = hexane/methanol/acetone (2:1:1; v/v/v/v), AW = acetone/water/acetic acid (70:29.5:0.5; v/v/v).

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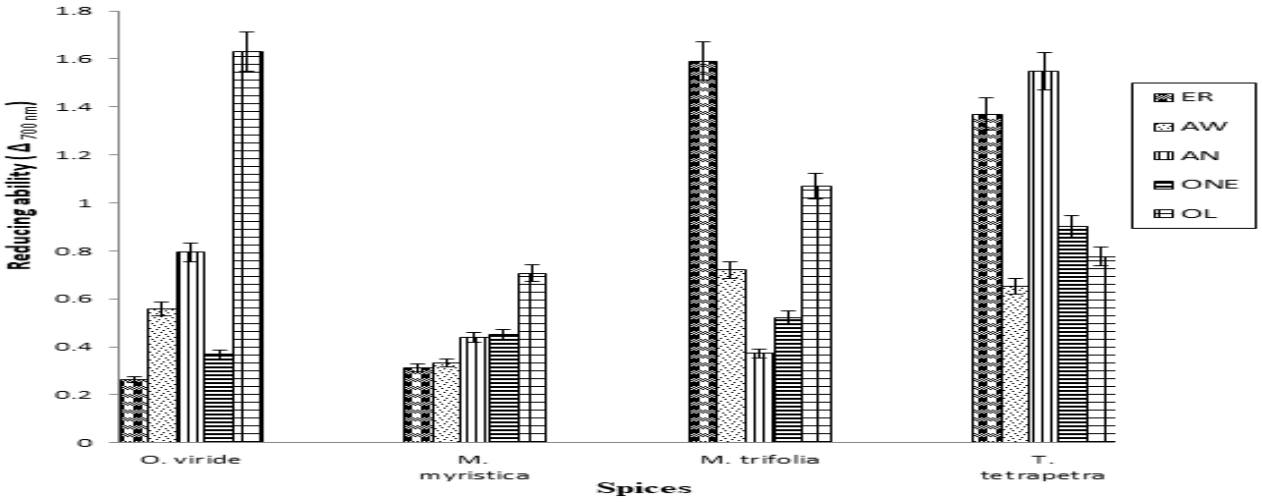

Fig. 1:- Comparative reducing power of spice extracts from different solvents. ER = distilled water, OL = 95% methanol, ONE = acetone/hexane (1:1; v/v), AN = hexane/methanol/acetone (2:1:1; v/v/v/v), AW = acetone/water/acetic acid (70:29.5:0.5; v/v/v).

Monodora myristica exhibited the least reducing power (0.263 and 0.303 respectively) among the various solvent extracts of each spice whereas water extract of *Monodora tenuifolia* exhibited the highest reducing power (1.59) among the five solvent extracts of the spice. The relative differences in reducing power of various solvent extract of the four spices are conspicuously exhibited in the bar chart (Figure 1). Generally, methanol extracts of all the spices showed relatively high reducing power, with that of *Tetrapleura tetrapetra* exhibiting the highest reducing power when compared with methanol extract of the other spices. The spice and solvent characteristics and concentration of the spice in the solvents all affected reducing power of the spice extracts. Plant materials, including spices, with high reducing power are usually good natural antioxidants [10]. The reducing power of the four spices could be summarized in the following order from high to low: *T. tetrapetra>M. tenuifolia>O. viride>M. myristica.*

C. Inhibition of linoleic acid peroxidation by solvent extracts of spices

Figure 2 shows the percentage inhibition of linoleic acid of various solvent extracts of the spices at 20 mg dry matter/ml, using ferric thiocyanate method (FTC). Ferric thiocyanate method estimates the ability of the antioxidant compounds to suppress pro-oxidant and oxidant activities in oxidizing systems. It is most appropriate at the initial stage of lipid peroxidation, and in estimating delay or prevention of low-density lipoprotein (LDL) peroxidation by antioxidants (Hwang *et al.*, 2013). Delay or prevention of linoleic acid peroxidation is an indication of antioxidant activity [11]. A higher percentage inhibition indicates higher antioxidant activity. All the solvent extracts except water extract of *M. tenuifolia* exhibited more than 50 % linoleic acid peroxidation.

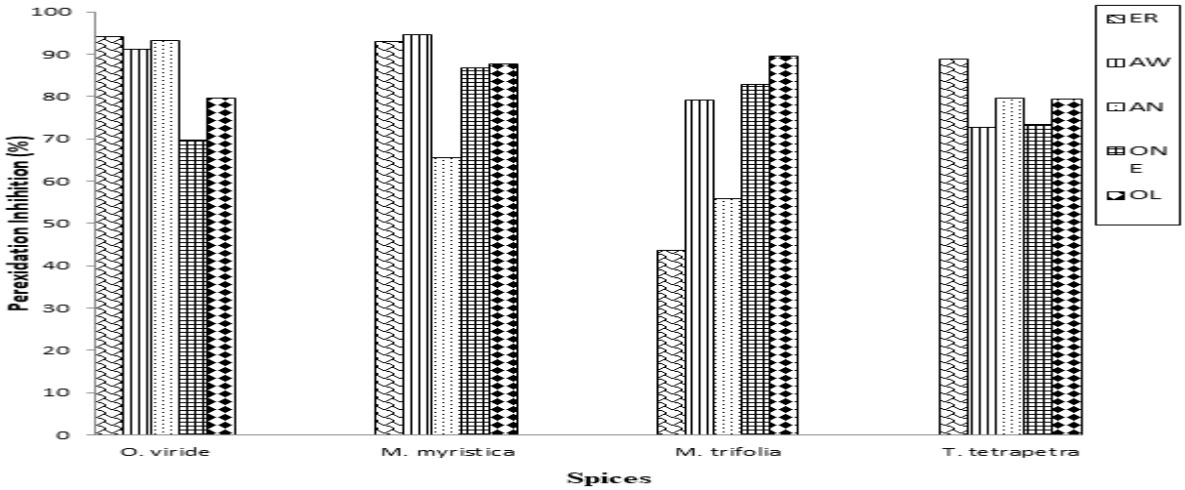

Fig. 2: Inhibition of linoleic acid per oxidation by different solvent extracts of spices.ER = distilled water, OL = 95% methanol, ONE = acetone/hexane (1:1; v/v), AN = hexane/methanol/acetone (2:1:1; v/v/v/v), AW = acetone/water/acetic acid (70:29.5:0.5; v/v/v).

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The ranges of peroxidation inhibition (%) were 43.5 for water extract to 89.5 for 95 % methanol extract of *Monodora tenuifolia*, 72.6 for acetone/water/acetic acid extract to 88.70 for water extract of *Tetrapleura tetrapetra*, 65.5 for hexane/methanol/acetone extract to 94.6 for water extract of *M myristica*, and 69.6 for acetone/hexane extract to 94.13 for water extract of *Ocimum viride*. Water extracts of *Ocimum viride* and *Tetrapleura tetrapetra*, acetone/water/acetic acid extract of *Monodora myristica* and 95% methanol extract of *Monodora tenuifolia* significantly ($P<0.05$) exhibited highest percentage linoleic acid inhibition for each spice.

D. Scavenging of 1,1 – diphenyl-2-picryl Hydrazyl Radical (DPPH) by the Spices

The scavenging potentials of various solvent extracts of the four spices against DPPH radical is shown in Table 2. The five solvent extracts had exceptionally high scavenging activity suggesting that the spices have good radical scavenging constituents. The extracting solvents affected radical quenching capacity of the spices. Generally, a stronger radical quenching agent has a lower IC_{50} value [16]. The water extracts of *M. myristica* (3.29 mg/g) and *T. tetrapetra* (3.78 mg/g), hexane/methanol/acetone extract of *M. tenuifolia* (3.70 mg/g) and acetone/water/acetic acid extract of *O. viride* (3.64 mg/g) had the lowest IC_{50} among the five solvent extracts of each spice. Evidently, the IC_{50} of the four spices, regardless of type of solvent used, fall within a very close range of 3.29 to 3.78. Water alone and methanol in combination with other solvents were the best extracting solvents for radical scavenging antioxidant properties. An antioxidant scavenging capacity on 1, 1-diphenyl-2-picry1hydrazyl (DPPH) radical is based on its reduction and decolourization of its purple colour to reduced yellow coloured 2, 2-diphenyl-1-picry1hdrazine, a stable free radical, by an electron- or hydrogen-donating molecule, usually a free radical scavenging antioxidant [27]. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, that is a free radical scavenging antioxidant, the absorption strength decreases and this is quantified stiochiometrically with respect to the number of electrons captured. Because of its odd electron, 1, 1-diphenyl-2-picry1hdrazyl gives a strong absorption maximum at 527 nm with visible spectrophotometer (purple colour). An increase in the amount of antioxidant compounds present in the extracts result to an increase in the DPPH free radical scavenging activity, and lower absorbance values [29].

IC_{50} (mg/ml)				
Solvents	*Monodoramyristica*	*Monodoratenuifolia*	*Ocimumviride*	*Tetrapleura Tetrapetra*
ER	3.29^{c}±0.13	4.72^{a}±0.014	4.25^{c}±0.91	3.78^{d}±0.04
AW	3.63^{b}±0.11	3.80^{c}±0.11	3.64^{e}±0.71	3.91^{c}±0.20
AN	3.67^{b}±0.09	3.70^{d}±0.0	4.46^{b}±0.81	4.14^{b}±0.11
ONE	5.18^{a}±0.06	4.27^{b}±0.18	16.03^{a}±1.12	4.09^{b}±0.12
OL	3.63^{b}±0.02	4.33^{b}±0.09	3.93^{d}±0.011	4.57^{a}±0.07

Table 2:- Free radical 1,1-diphenyl-3-picryl hydrazy (DPPH) Scavenging activity by different solvent extracts of spices.

Data are means ± standard deviations (n = 3); values marked by the same letters within the same column are not significantly different ($p < 0.05$). ER = distilled water, OL = 95% methanol, ONE = acetone/hexane (1:1; v/v), AN = hexane/methanol/acetone (2:1:1; v/v/v/v), AW = acetone/water/acetic acid (70:29.5:0.5; v/v/v).

E. Comparative DPPH radical scavenging activity of various solvent extracts of spices

Figure 3 compares radical scavenging activities of the four spices in the five solvents at 25 mg/ml concentrations. *Tetrapleura tetrapetra* exhibited highest radical scavenging activity. The extracting solvents affected radical scavenging activities of the spices. Water extracts of *T. tetrapetra* and *M. myristica* had highest radical scavenging constituents of both spices. Scavenging activities (%) of the spices as affected by the extracting solvents ranged from 25 to 33.7 for *O. viride*, 29.3 to 35.5 for *M. myristica*, 15.0 to 35 for *M. tenuifolia* and 38.0 to 43.0 for *T. tetrapetra*. It is generally recognized that free radicals produced in the body are purely associated with etiology of cancers and other chronic diseases. Dietary antioxidants capable of scavenging free radicals can reduce the risks of cancer and chronic diseases [27], [1].

F. Inhibition of lipid peroxidation in cooked, ground meat patties by spices during storage

Table 3 compares the IC_{50}, which is the final concentration (mg/g) of the dry spice required to suppress lipid peroxidation in cooked, ground beef and pork by 50 %, using the thiobarbituric acid method. The thiobarbituric acid (TBA) method estimated degree of lipid peroxidation in the oxidizing pork and beef by quantifying the amount of malonaldehyde formed. The degree of deterioration of fatty foods is detected by the intensity of red pigmentation formed when TBA is reacted with oxidizing lipid. Thiobarbituric acid method is most sensitive and persistent than many other

known methods of antioxidant analysis simply because it quantifies the end-product which is stable for a period of time [15]. Higher IC_{50} value implies lower antioxidant activity [22], [28], [31]. The results showed that IC_{50} value in pork patties ranged from 1.08 to 2.30 mg/g while the value in beef patties ranged from 1.12 to 1.79 mg/g. *Ocimum viride*, *T. tetrapetra*, *M. myristica* and *M. tenuifolia* had lower IC_{50} in beef patties than in pork patties, indicating higher antioxidant properties of these four spices in beef than in pork. At the same use level (mg/g), *O. viride* and *T. tetrapetra* exhibited higher antioxidant properties than *M. myristica* and *O. trifolia* in both pork and beef patties.

IC_{50} (mg/g)		
Spices	Pork	Beef
Ocimum viride	$1.80^{a}\pm0.04$	$1.36^{b}\pm0.06$
Monodora myristica	$2.08^{a}\pm0.09$	$1.22^{bc}\pm0.04$
Monodora tenuifolia	$2.30^{a}\pm0.09$	$1.79^{a}\pm0.21$
Tetrapleuva tetrapetra	$1.53^{b}\pm0.11$	$1.12^{c}\pm0.12$

Table 3:- Inhibition of lipid peroxidation in cooked, ground meat patties by spices

Data are means ± standard deviations (n = 3); values marked by the same letters within the same column are not significantly different ($p < 0.05$).

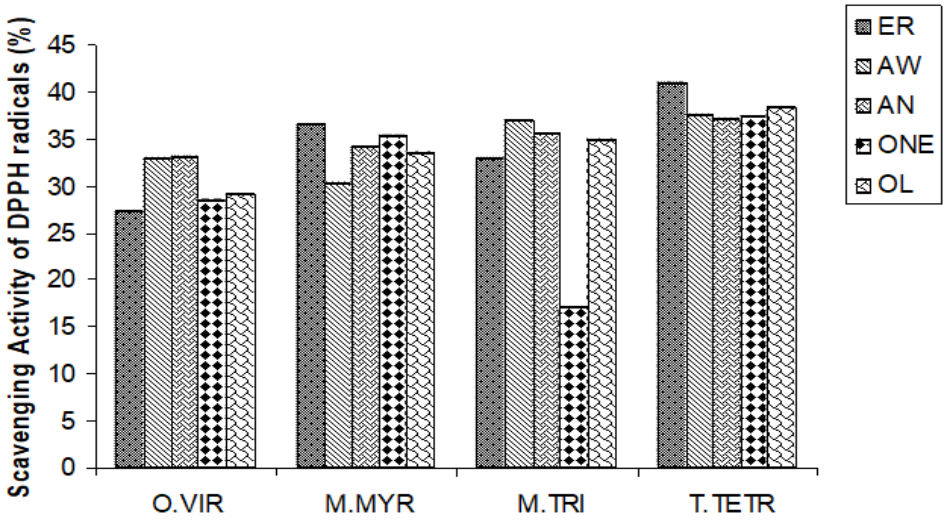

Fig. 3:- DPPH radical scavenging activities of different solvent extracts (mg dry matter/mil) of *Ocimum viride* (O. vir), *Monodora myristica* (M.myr), *Monodora tenuifolia* (M. tri) and *Tetrapleura tetrapetra* (T. tetr). Values are means of triplicate determinations.ER = distilled water, OL = 95% methanol, ONE = acetone/hexane (1:1; v/v), AN = hexane/methanol/acetone (2:1:1; v/v/v/v), AW = acetone/water/acetic acid (70:29.5:0.5; v/v/v).

IV. CONCLUSION

In this present study, the four Nigerian spices possess phenols and exhibited dose-dependent antioxidant properties which were influenced by extracting solvents. Spices reduced F^{e3+} to F^{e2+}, scavenged DPPH radical, inhibited linoleic acid peroxidation in oxidizing FTC systems and inhibited formation of thiobarbituric reactive substances in cooked ground beef and pork patties during storage. These spices could act as natural antioxidants in real food systems and be exploited to manage radical-related degenerative diseases in human. Spice antioxidant was in the following order from high to low: *T. tetrapetra>O. viride>M. myristica>M. tenuifoli.*

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