

SJMLS-3(1)-010

**Differential Solvent Extraction on The Antioxidant Properties, and Phytochemical Compounds of *Monodora myristica*****Okpoghono, J.\* , Achuba, F.I. and George, B.O.**Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Delta State, Nigeria<sup>1</sup>  
Author for Correspondence\*: [okpoghono@gmail.com](mailto:okpoghono@gmail.com)/+234-806-461-8605**Abstract**

Biochemical investigations were carried out to reveal the solvent differential as it affects the antioxidant properties and gas chromatography mass spectrometry (GC-MS) analysis of *Monodora myristica*. The *M. myristica* seeds were sun-dried for two weeks to obtain constant weight and then crushed into fine particles using electric blender. Extraction was done using water (60 °C), ethanol and diethyl ether. Biochemical analyses were carried out using standard methods. The results of the phytochemical screening of *M. myristica* crude sample indicate the presence of saponin (4.25 %), tannin (0.53 %), alkaloid (3.82 %), flavonoid (3.47 %). The total phenolic content, ascorbate oxidase and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *M. myristica* extracts were as follows; *M. myristica* diethyl ether extract (MDEE) > *M. myristica* ethanol extract (MEE) > *M. myristica* water extract (MWE). The gas chromatography mass spectrometry (GCMS) analysis showed the presence of twelve compounds in MDEE, eight compounds from MEE and six compounds were obtained from MWE. It could be concluded that *M. myristica* seeds possess antioxidant properties and contains various phytochemical compounds. This may confirm the traditional uses of *M. myristica* seeds as an antioxidant of nutritional significance.

**Key words:** *Monodora myristica*, Antioxidant, Water, Ethanol, Diethyl ether.

**Introduction**

Spices are products of plants which are used in

various forms for food preparation (Parveen et al., 2014). They have been used for centuries by many cultures to enhance flavor, aroma and as preservatives (Ene-obong et al., 2015). Otunola et al. (2014) reported that the prevention of certain degenerative diseases like cancer and cardiovascular diseases has been associated with the ingestion of spices, fresh fruits and vegetables rich in natural antioxidative compounds. Spices and herbs with different medicinal properties are used therapeutically to treat various undesirable clinical conditions (Hart, 2005).

Solvent extraction is a technique that is often used for the isolation of antioxidant compounds from plant (Peschel et al., 2006). The extract yield with resultant antioxidant activities of the plant is strongly dependent on the extracting solvents. This may be due to different antioxidant compounds present in the plant with varied chemical polarities in a particular solvent (Peschel et al., 2006).

Varieties of chemical compounds produced by plant can be analyzed using qualitative phytochemical screening and quantification of the metabolites may help to extract, purify and then identify the bioactive compounds. Gas chromatography mass spectrometry is a compatible technique that is used mainly for identification and quantification purpose. The complex mixture of unknown organic compounds can be determined by interpretation or matching the spectra with reference spectra (Hites, 1997).

The spice *M. myristica* belongs to the custard apple family of flowering plants called Annonaceae. It is widely distributed from Africa to Asia, America and Australia (Omobuwajo *et al.*, 2003). The seeds of *M. myristica* are aromatic and are used after grinding to a powder as condiments in food preparation (Ekeanyanwu

*et al.*, 2010). Figure 1, showed the picture of *M. myristica* seeds. The aim of this study was to reveal the differential in extraction solvents (water, ethanol and diethyl ether) as they affect the antioxidant properties, and bioactive compounds of *M. myristica*



**Figure 1.** *M. myristica* seeds

## Materials and Methods

### Spice (*Monodora myristica*)

The spice *M. myristica* was purchased from the local market in Obiaruku, Delta State, Nigeria. The spice was identified at the Department of Botany, Delta State University, Abraka, Delta State, Nigeria.

### Preparation of the Spice Extracts

The extract of *M. myristica* was obtained using the extraction technique as previously described by George *et al.* (2012). Briefly the spice was sun-dried to constant weight for two weeks and then crushed into fine particles using electric blender at high speed. One hundred grams (100g) of the powdered spice was extracted with 500 ml of the respective solvent (hot water (60°C), ethanol (95 % v/v), and diethyl ether, 95 % v/v) and allowed to stand for 48 hrs. The mixture was then filtered using a clean muslin

cloth. Thereafter the filtrate was evaporated to dryness using rotary evaporator attached to a vacuum pump. The percentage yield of each extract was determined for each solvent. The extracts were stored in refrigerator until needed for further analysis

$$\text{Percentage yield (\%)} = \frac{\text{Dry weight (extract)}}{\text{Dry sample weight}} \times 100$$

Dry sample weight 1

Qualitative and Quantitative Phytochemical Study: Qualitative phytochemical study was carried out using the method described by Nwokonkwo (2013). Each extract (0.5 g) was dissolved in 20 cm<sup>3</sup> of the corresponding solvent (water, ethanol and diethyl ether), and set aside for qualitative phytochemical analysis. The quantitative analysis was carried out using the method of Harborne (1973).

**Antioxidants Analysis:** Determination of total phenolic content in the spice extracts was carried out according to the method described by Liu and Yao (1997). Total flavonoid of the spice extracts was determined with colorimetric aluminum chloride methods as described by Ebrahimzadeh *et al.* (2008). The free radical scavenging ability of the sample extracts against DPPH (2, 2-diphenyl-1 picrylhydrazyl) free radical was estimated using the method described by Ursini *et al.* (1994). The assay of ascorbate oxidase activity in the spice extracts were carried out using the method of Vines and Oberbacher (1965).

**Gas chromatography mass spectrometry analysis:** Gas chromatography mass spectrometry was carried out according to the method of Dhivya and Manimegalai (2013). The Identification of constituents was based on comparison of constituent of analyst with National Institute of Standards and Technology (NIST) Libraries using mass Hunter Software.

### Statistical Analysis

Descriptive statistics was carried on the data obtained and results were expressed as mean  $\pm$ SD. The SPSS-PC program package (version 22.0) was used for statistical analysis. A significant threshold of  $p \leq 0.05$  was regarded statistically significance between the test and control group for the analysis.

### Results

The percentage yield of the water, ethanol and diethyl ether extracts of the powdered seeds of *M. myristica* (Table 1) showed that water had 10.84 %, ethanol 19.82 % and diethyl ether 27.90 %. Table 2 and 3 phytochemicals screening revealed the presence of saponin, tannin, alkaloid and flavonoid.

**Table 1. Percentage yield of the crude extracts of *M. myristica* seeds**

Extracting solvent	Seed powder (g)	Extracted seed powder (g)	Yield (%)
Water	100	10.80	10.80
Ethanol	100	19.82	19.82
Diethyl ether	100	27.90	27.90

**Table 2. Qualitative phytochemical screening of *M. myristica* crude sample**

Phytochemical	Percentage yield
Saponin	4.25 %
Tannin	0.53 %
Alkaloid	3.82 %
Flavonoid	3.47 %

**Table 3. Quantitative phytochemical screening of *M. myristica* in different solvents.**

Phytochemicals	MWE	MEE	MDEE
Saponin	+	+	+
Tannin	+	+	+
Alkaloid	+	+	+
Flavonoid	+	+	+
Phlobatannins	-	-	-

+= Present

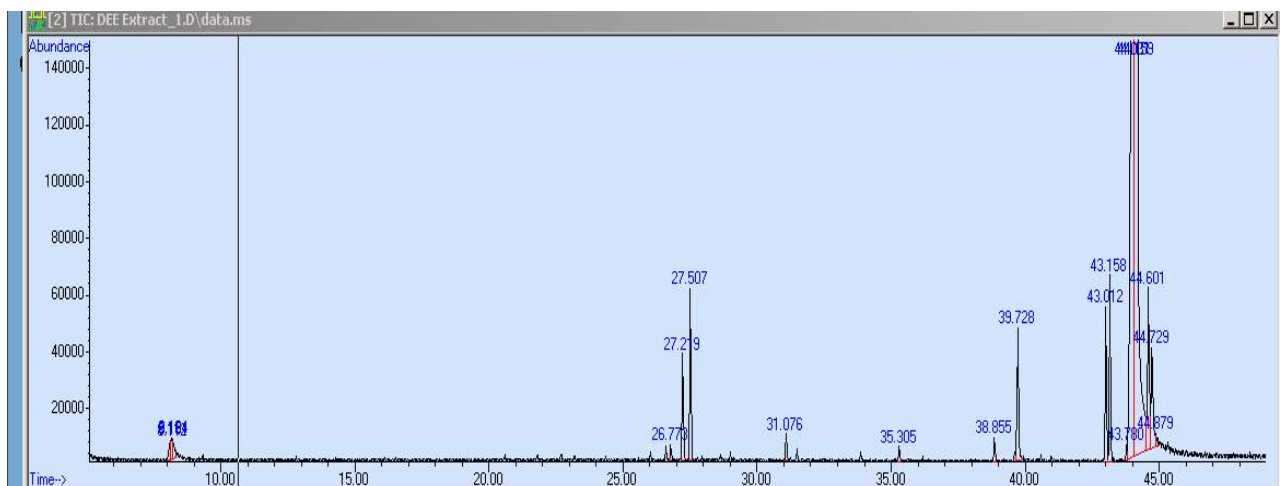
-= Absent

NQ = Not Quantified

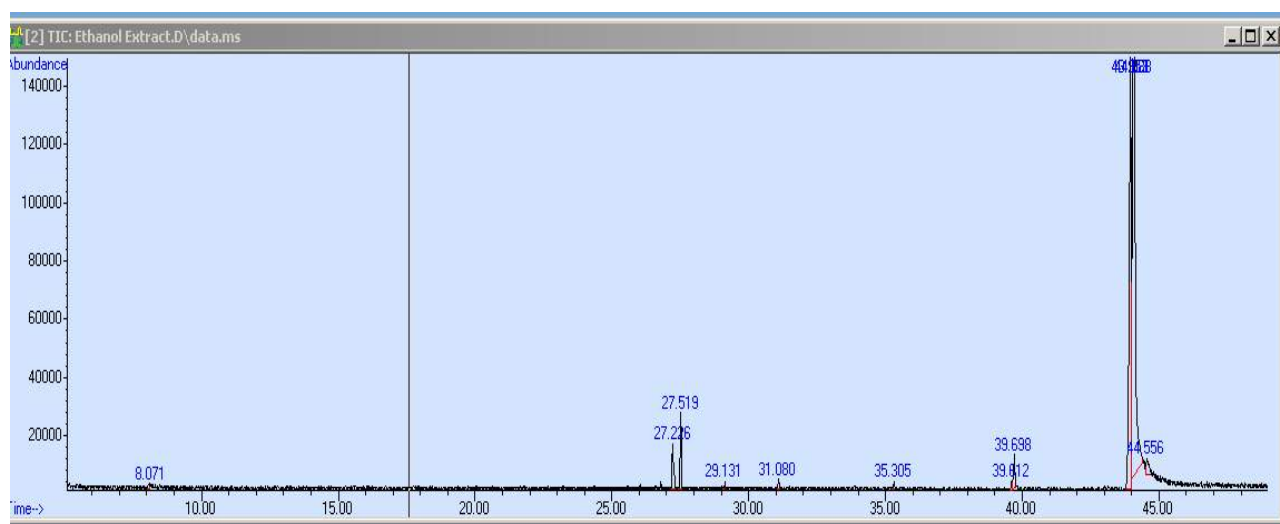
**Table 4: Antioxidant activities of *M. myristica* different extracts .**

Parameters	Concentrations of extracts	MWE	MEE	MDEE
<b>Total phenolic content (units/ml)</b>				
	25 µg/ml	10.30 ± 4.19 <sup>a</sup>	25.36 ± 2.03 <sup>b</sup>	30.45 ± 4.10 <sup>c</sup>
	50 µg/ml	12.30 ± 3.45 <sup>a</sup>	30.30 ± 2.23 <sup>b</sup>	41.91 ± 2.50 <sup>b</sup>
	100 µg/ml	15.23 ± 4.23 <sup>a</sup>	44.30 ± 5.56 <sup>a</sup>	53.23 ± 12.0 <sup>a</sup>
<b>Total Flavonoid (units/ml)</b>				
	25 µg/ml	15.32 ± 0.95 <sup>a</sup>	25.35 ± 2.13 <sup>b</sup>	37.16 ± 6.93 <sup>c</sup>
	50 µg/ml	22.34 ± 4.10 <sup>b</sup>	31.23 ± 5.34 <sup>c</sup>	51.34 ± 2.12 <sup>a</sup>
	100 µg/ml	34.23 ± 5.23 <sup>c</sup>	46.32 ± 8.90 <sup>a</sup>	62.23 ± 10.23 <sup>b</sup>
<b>Ascorbate oxidase activity (units/ml)</b>				
	25 µg/ml	0.73 ± 0.12 <sup>a</sup>	0.41 ± 0.09 <sup>a</sup>	0.92 ± 0.22 <sup>a</sup>
	50 µg/ml	1.00 ± 0.01 <sup>a</sup>	0.90 ± 0.03 <sup>a</sup>	1.85 ± 0.03 <sup>a</sup>
	100 µg/ml	1.90 ± 0.03 <sup>a</sup>	1.15 ± 0.05 <sup>a</sup>	2.55 ± 0.09 <sup>a</sup>
<b>DPPH radical scavenging activity (%)</b>				
	25 µg/ml	30.41 ± 5.35 <sup>a</sup>	40.34 ± 4.06 <sup>b</sup>	58.30 ± 14.81 <sup>c</sup>
	50 µg/ml	40.45 ± 5.90 <sup>b</sup>	54.98 ± 8.91 <sup>c</sup>	60.54 ± 13.44 <sup>c</sup>
	100 µg/ml	56.40 ± 12.80 <sup>c</sup>	68.88 ± 21.35 <sup>a</sup>	85.85 ± 10.67 <sup>b</sup>

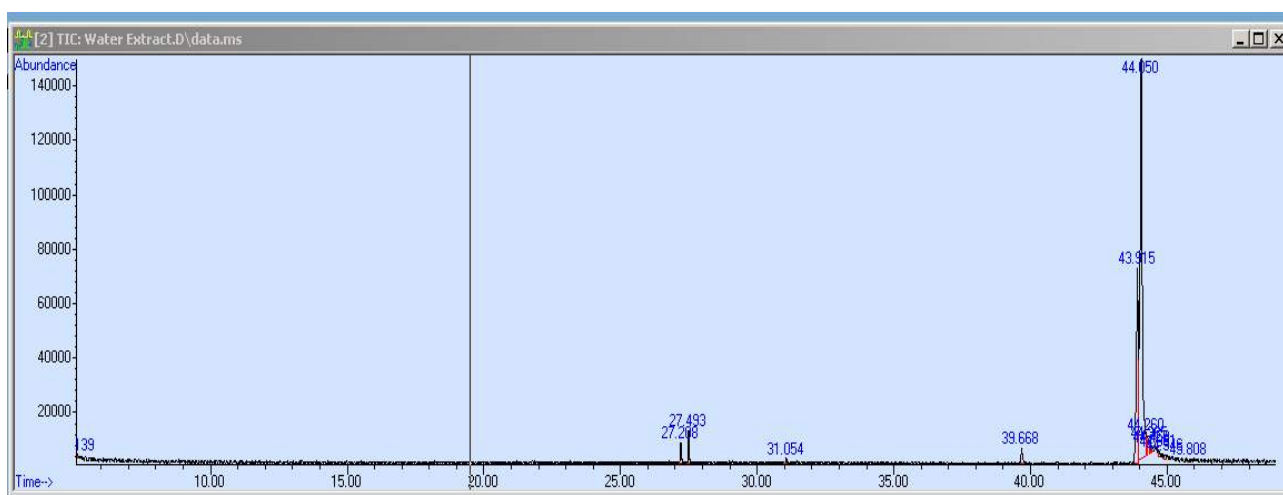
Triplicates values are represented in mean ±SD Mean values with different superscript letter in the same horizontal row differ significantly at  $p < 0.05$ .



**Figure 1: GC-MS chromatogram of MDEE.**

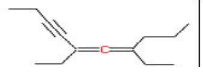
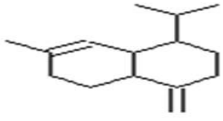
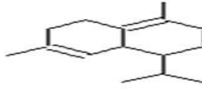

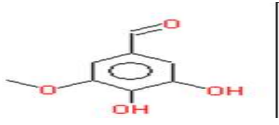
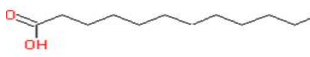
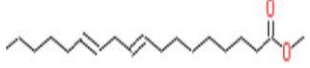


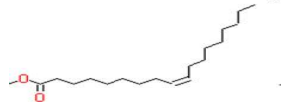


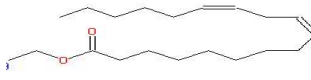
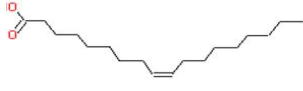
**Figure 2: GC-MS chromatogram of MEE.**

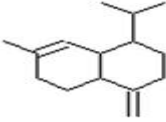
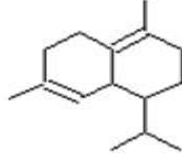
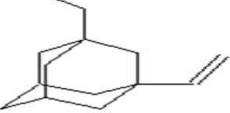
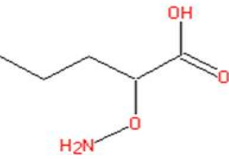
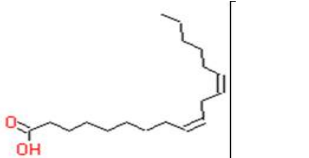
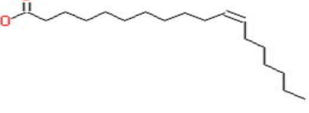


**Figure 3: GC-MS chromatogram of MWE.**

**Table 5. Phytochemical Compounds Identified in the Diethyl Ether Extracts of *M. myristica* Showing Compound Nature, Molecular Formula and Structures.**

<i>M. myristica</i> Diethyl Ether Extract						
No.	RT (min)	MW	Names	Formula	Compound nature	Structure
1.	26.780	190	5,6-decadien-3-yne,5,7-diethyl	C <sub>14</sub> H <sub>22</sub>	Alkene, Alkyne	
2.	27.222	240	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)	C <sub>15</sub> H <sub>24</sub>	Sesquiterpene	
3.	27.511	204	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl) -, (IS cis)	C <sub>15</sub> H <sub>24</sub>	Sesquiterpene	
4.	31.076	222	1-Naphthelenol,1,2,3,4,4a,7,8,8a-octahydro-1,6-methyl-4-(1-methylethyl)-	C <sub>15</sub> H <sub>26</sub> O	Sesquiterpene Alcohol	
5.	35.305	168	3,4,-Dihydroxy-5-methoxybenzaldehyde	C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>	Aromatic Aldehyde	
6.	39.728	256	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	carboxylic acid	
7.	43.012	294	9,12-octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	Unsaturated fatty acid ester	

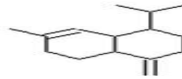
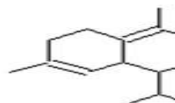
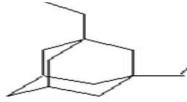
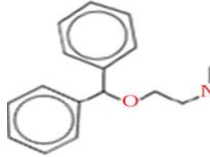
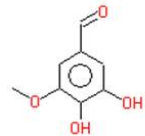
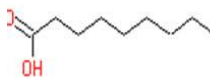
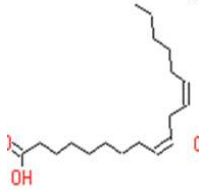
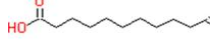
8.	43.158	296	9-Octadecenoic acid (Z) -methyl ester	$C_{19}H_{36}O_2$	Unsaturated fatty acid ester	
9.	44.009	280	9,12-Octadecadienoic acid (ZZ)-	$C_{18}H_{32}O_2$	Double unsaturated mega-6 fatty acid (carboxylic acid)	
10.	44.136	282	Cis Vaccenic acid or (Z)-11 octadecenoic acid)	$C_{18}H_{34}O_2$	Monounsaturated omega -7 fatty acid (carboxylic acid)	
11.	44.605	308	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	Unsaturated fatty acid ester	
12.	44.732	282	Oleic acid	$C_{18}H_{34}O_2$	Monounsaturated omega -9 fatty acid (carboxylic acid)	

<i>M. myristica</i> Water Extract						
No.	RT (min)	MW	Names	Formula	Compound nature	Structure
1.	27.204	204	Naphthalene 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl-	$C_{15}H_{24}$	Sesquiterpene	
2.	27.496	204	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-(IS cis-	$C_{15}H_{24}$	Sesquiterpene	
3.	31.054	190	1-Ethyl-3-vinyl-adamatane	$C_{14}H_{22}$	Cycloalkane	
4.	39.676	133	Pentanoicacid, 2-(aminoxy)-	$C_5H_{11}O_3$	Carboxylic acid	
5.	43.912	280	9,12-octadecadienoic acid (ZZ)-	$C_{18}H_{32}O_2$	Double unsaturated omega-6 fatty acid (carboxylic acid)	
6.	44.047	282	Cis Vaccenic acid or (Z)-11-octadecenoic acid)	$C_{18}H_{34}O_2$	Monounsaturated omega-7 fatty acid (carboxylic acid)	

**Table 6. Phytochemical Compounds Identified in the Water Extracts of *M. myristica* Showing Compound Nature, Molecular Formula and Structures**

**Table 7. Phytochemical Compounds Identified in the Ethanol Extracts of *M. Myristica* Showing Compound Nature, Molecular weight, Molecular Formula and Structures.**



<i>M. myristica</i> Ethanol Extract						
No.	RT (min)	MW	Names	Formula	Compound nature	Structure
1.	27.226	204	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methyl-4-methylene 1-(1-methylethyl)-	C <sub>15</sub> H <sub>24</sub>	Sesquiterpene	
2.	27.519	204	Naphthalene, 1,2,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl) (IS cis)-	C <sub>15</sub> H <sub>24</sub>	Sesquiterpene	
3	31.084	190	1-Ethyl-3-vinyl-Adamantane	C <sub>14</sub> H <sub>22</sub>	Cycloalkane	
4	35.297	241	N-Desmethyl diphenhydramine	C <sub>16</sub> H <sub>19</sub> N O	Amine	
5	35.315	168	3,4-Dihydroxy-5-methoxy-benzaldehyde	C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>	Aldehyde	
6	39.709	256	N Hexadecanoic Acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	carboxylic acid	
7	43.150	280	9,12-octadecadienoic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	Double unsaturated omega-6 fatty acid (carboxylic acid)	
8	44.084	282	Cis Vaccenic acid or (Z)-11-octadecenoic acid)	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	Monounsaturated omega-7 fatty acid (carboxylic acid)	

## Discussion

The percentage yields of *M. myristica* as shown in Table 1, were higher than those obtained from a previous study (Enabulele and Ehiagbonare, 2011). However, in another plant, *Perinary excels*, the percentage yields for water was 8.45 % and ethanol 6.32 %. The yields are also higher than those obtained from *M. myristica* seeds extract reported by Enabulele *et al.* (2014), water 7.94 %, ethanol 12.5 % and diethyl ether extract percentage yield was not determined. El-Mahmood *et al.* (2008) stated that factors like type of plant material, age of plants and polarity of the solvent used could affect the extract percentage yield. Polarity of solvents (type of solvent) plays a vital role in extraction process. Solvent polarity may have influence on the ability to dissolve a special group of antioxidant compounds (phenolic compounds) and influences the antioxidant activity estimation. Study has shown that mixing diethyl ether and water, the diethyl ether layer is on top due to the fact that it has a lower density than the water thus less polar than ethanol and water (Adrian, 2010). In this study, diethyl ether as an extracting solvent was seen to be better than ethanol and water as the yield was almost twice that for ethanol and water.

In Tables 2 and 3, the observed saponin, tannin, alkaloid and flavonoid in the different extracts of *M. myristica* were in line with the results of Erukainure *et al.* (2012), who reported the protective potentials of antioxidants spices against free radical *in vitro*. Njoku and Akumefula (2007), reported that alkaloids are the most efficient therapeutically significant phytochemical. They are widely used as cancer chemotherapeutic agents (Stray, 1998). The tannin, flavonoid and the phenolic contents may contribute to the antioxidant activities of spices (Erukainure *et al.*, 2012). Studies have shown that free or protein complex condensed and hydrolysable tannins are more effective than phenolics (Erukainure *et al.*, 2012). Saponin serves as natural antibiotics which may help to fight infections and microbial invasions in the body (Nijveldt *et al.*, 2001).

The results indicated that *M. myristica* have high levels of total phenol and flavonoid contents with

antioxidative effects (Table 4). This effect was more pronounced in MDEE when compared with MEE and MWE. Ademiluyi and Obboh (2008) reported that phenols and flavonoids act as powerful antioxidants that protect the human body against free radicals. Phenolics antioxidant activity may be due to their redox properties that allow them to act as free radical quenchers, metal chelators and also as reducing agents (Erukainure *et al.*, 2012; Rice-Evans *et al.*, 1996). In this study ascorbate oxidase activities were present in the three extracts and were comparable (MDEE > MEE > MAE). Previous study conducted by Foyer (1983) reported that ascorbate act as a reductant for many free radicals. Ascorbate oxidase is a multi-copper enzyme which catalyzes the oxidation of ascorbic acid to dehydroascorbic acid. Ascorbate oxidase participates in the redox system involving ascorbic acid (Pignocchi *et al.*, 2003). Also, 2,2 – dipheny -1-picrylhydrazyl (DPPH) was applied to evaluate the radical scavenging ability of antioxidants (Brad-Williams *et al.*, 1995). *M. myristica* extracts were observed to exhibit DPPH scavenging activity. The high phenolic and flavonoid contents of *M. myristica* extracts could have be responsible for the observed high scavenging activity. This is in line with the reports of Zhou and Yu (2006), that correlated total phenolic content with the DPPH radical scavenging activity in vegetable extracts, therefore making suggestion that total phenolics plays a major role in the antioxidant activity of plant materials. Studies have also suggested that under certain physiological conditions (high concentrations of phenolic antioxidants) initiate an auto-oxidation process, therefore acting like pro-oxidants (Erukainure *et al.*, 2012; Halliwell, 2008).

Comparison of the mass spectrum in the different extracts of *M. myristica* with the National Institute of Standards and Technology (NIST) database library gave confirmatory compound structure match. The compounds were classified based on their functional group (Tables 5, 6 and 7). Functional hydroxyl groups mediate their antioxidant properties by scavenging free radicals or by chelating metal ions (Kumar and Pandey, 2013). Kumar *et al.* (2013) reported that chelation of metals could be of importance in the

prevention of radical generation which may lead to damage of target biomolecules. This work helps to identify the bioactive compounds, that have antioxidant and anti-inflammatory properties which may be used in body products, pharmaceutical and therapeutic values. Cis-Vaccenic acid which was found in the three extracts had been reported to have antioxidant and anti-inflammatory properties (Bhattacharya *et al.*, 2014).

### Conclusion

The high antioxidant ability observed in MDEE than MEE and MWE, may be due to the presence of the following compounds; alkene and alkyne (5,6-decadien-3-yne, 5,7, diethyl), sesquiterpene alcohol (naphthelenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-methyl-4-(1-methylethyl-), fatty acid esters (9,12-octadecadienoic acid), and methyl ester (9-octadecenoic acid (Z)-methyl ester; and linolenic acid ethyl ester) which are not found in MEE and MWE. That MEE possesses the ability to act as an antioxidant than MWE, may be due to the fact that the extract contains the following compounds; Aldehyde (3,4-Dihydroxy-5-methoxy-benzaldehyde) and amine (N-Desmethyl-diphenhydramine) which are not present in MWE. The compounds obtained from *M. myristica* extracts contains carbonyl group, hydroxyl group and double/triple bonds conjugates which ideally are able to give off hydrogen and become a radical without losing much stability. The results of this study confirmed the traditional uses of *M. myristica* seeds as an antioxidant of nutritional significance and may be recommended as a spice of phytopharmaceutical importance.

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**Citation:** Okpoghono, J., Achuba, F.I. and George, B.O. Differential Solvent Extraction on The Antioxidant Properties, and Phytochemical Compounds of *Monodora myristica*. *Sokoto Journal of Medical Laboratory Science*; 3(1): 88 - 100.

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