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Research Article

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Lipid compositions of three local spices commonly consumed in Ekiti state, Nigeria

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Abstract The study is aimed to isolate and analyse the lipids (fatty acids, phospholipids and phytosterols) of three plant products commonly used as food ingredients (*Bridelia ferruginea* stem bark (Ira), *Monodora myristica* seed kernel (Ariyo) and *Zingiber officinale Roscoe* (Ajo)). The fat (%), total fatty acid (%) and energy (kJ/100g) had range values of 1.40 - 30.5, 1.12 - 24.4 and 41.4 - 903 respectively. Saturated fatty acids (SFA) ranged from 17.2 - 46.2 %, total monounsaturated fatty acids (MUFA) values ranged from 20.2 - 51.5, n-3 and n-6 fatty acids values ranged from 0.724 - 2.50 and 26.4 - 61.4 respectively. Phospholipids level was averagely high at total values of 110 - 263 mg/100g. Among the phytosterols, sitosterol was the most concentrated (28.2 - 69.0 mg/100g) within a total value of $37.5 \cdot 102$ mg/100g. Chi-square (X^2) analysis showed that significant differences existed at $\alpha_{=0.05}$ among the following parameters: SFA, MUFA*cis*, total MUFA, n-6 polyunsaturated fatty acid (PUFA), n-6/n-3, LA/ALA, Phosphatidylethanolamine, phosphatidylcholine, Phosphatidylserine, Phosphatidylinositol, Stigmasterol, 5-Avenasterol and sitosterol. The analyzed plant samples were averagely low in total fatty acids, phospholipids and phytosterols, hence their consumption, as food sources either as spice or soup ingredients may not result in the consumers consuming fats above the recommended healthy guidelines.

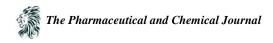
Keywords local spices, fatty acids, phospholipids, phytosterols composition

Introduction

Spices are the bark, seed, resin, root, stem, fruit, or bud of a plant, tree, or shrub. They count amongst their rank the familiar, such as *cinnamon*, *mustard*, *ginger*, *licorice*, *juniper*, and *cloves*. Also included are the strange and exotic, including *asafetida*, *nigella*, *silphium*, and *grains of paradise*. Many can be found at your neighbourhood market, and some can only be obtained on the other side of the globe [1]. Spices can be added to foods in several forms: as whole spices, as ground spices, as essential oils, as oleoresins or as prepared and filtered vinegar infusions. A more recent alternative is spice extracts. These consist of the flavour components of a spice, dispersed on one of several types of base, the most suitable bases for pickle and sauce use, for example, being salt or dextrose. Natural materials used in flavour creations are still most often isolated from essential oils [2].

In the South-western part of Nigeria especially, Ekiti State, people are fond of using a number of spices and herbs which include *Bridelia ferruginea* Benth stem bark, *Monodora myristica* seeds kernel and *Zingiber officinale Roscoe* (Ajo) as part of their everyday meal, cuisines and as medicine.

Bridelia ferruginea Benth (Euphorbiaceae) is a woody shrub which grows in the Savannah or rain forest of Africa [3]. In Ekiti State, it is popularly called Ira. The powdered stem bark has often been used in soap and food preparation. Traditionally, B. Ferruginea stem bark extracts is used as a milk coagulant, mouth wash, purgative, vermifugel and for the treatment of diabetes, arthritis, and boils [4]. Extracts of the plant have been shown to have molluscidal effect [4], antibacterial activity [6]. Flour of the stem bark has been reported to contain essential minerals and amino acids [7]. Monodora myristica is a species of calabash nutmeg usually called Ariyo in Yoruba. The edible seeds yield a nutmeg flavored oil which is used in West Africa for cooking [8]. Monodora myristica seed



extract had been reported to contain important pharmacological compounds like alkaloids, flavonoids, vitamin A and E as well as many important lipids [9]. *Zingiber officinale Roscoe* (Ajo) rhizome has been extremely popular for cooking as spice and to treat a host of ailments. It is a flowering plant in the family *Zingiberaceae* which has been reported to contain volatile oils and terpenoids [10].

In order to facilitate wider application of these plant materials as spice and soup ingredients, it is necessary to examine their nutritional compositions. To this end, this research work was conducted to determine the lipid (fatty acids, phospholipids and sterols) compositions of the flours of *B. ferruginea* Berth stem bark, *Monodora myristica* seeds kernel and *Zingibe officinale Roscoe* (Ajo).

Materials and Methods

Materials

Collection and treatment of samples

The samples were collected from local farms in Awo-Ekiti and Ado-Ekiti, Ekiti State. The samples were properly identified at the Department of Plant Science, Faculty of Science, Ekiti State University, Ado-Ekiti. They were washed, air dried and ground into fine flour.

Methods

Oil extraction

The fine flour of the three different samples were subjected individually to solvent extraction for 5 h with petroleum spirit between 40-60 °C boiling ranges using Semi-continuous Solvent extraction method (Soxhlet method) [11].

Preparation and analyses of fatty acid methyl ester (FAME)

The crude extraction was converted to the methyl ester using the boron trifluoride method [11]. The gas chromatographic conditions for the analyses of FAME (fatty acid methyl esters) were as follows: The GC was the HP 5890 powered with HP ChemStation rev A09.01 [1206] software [GMI, Inc, Minnesota, USA] fitted with a flame ionization detector (FID). A split injection with split ratio of 20:1 was used. GC inlet temperature was 250 °C with an oven programme of initial temperature at 60 °C, first ramping at 10 °C /min for 20 min (maintained for 4 min), second ramping at 15 °C/min for 4 min (maintained for 10 min) and detector temperature at 320 °C. A capillary column (30 m x 0.25 mm) packed with a polar compound (HP INNOWAX) with a diameter (0.25 μ m) was used to separate the esters. The peaks were identified by comparison with standard fatty acid methyl esters. Carrier gas used was nitrogen.

Sterol analyses

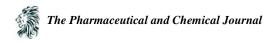
The sterol analysis was as described by AOAC [11]. The aliquots of the processed fat were added to the screw capped test tubes. The samples were saponified at 95 $^{\circ}$ C for 30 min, using 3 ml of 10 % KOH in ethanol, to which 0.20 ml of benzene had been added to ensure miscibility. Deionised water (3 ml) was added and 2 ml of hexane was added in extracting the non saponifiable materials. The extractions, each with 2 ml of hexane, were carried out for 1 h, 30 min and 30 min respectively. The hexane was concentrated to 1 ml in the vial for gas chromatography analysis and 1 μ l was injected into the injection pot of GC. The GC conditions of analyses were similar to the GC conditions for methyl esters analyses. The peaks were identified by comparison with standard sterols.

Phospholipids analyses

The method of Raheja *et al.* [12] was adopted in the analyses of the phospholipids content determination. The GC conditions for analyses of phospholipids were similar to FAME analyses except in the following: Column type was HP5, oven programme initial temperature at 50 °C, second ramping at 15 °C/min for 4 min, maintained for 5 min and the detector was pulse flame photometric detector (PFPD).

Quality assurance

For the purpose of ensuring the accuracy of the results obtained, the followings were prepared for sterols, phospholipids and fatty acid methyl esters which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient determined for fatty acids, sterols and phospholipids. Correlation is a statistical index that shows the quality assurance of the calibration curve performed.



It was prepared with the Hewlett Packard Chemistry (HPCHEM) software (GMI, Inc 6511 Bunker Lake Blvd Ramsey, Minnesota, 55303, USA).

Calculation of fatty acids per 100 g in sample as food

At the data source and reference database levels, values for individual fatty acids (FAs) are usually expressed as percentages of total FAs. At the user database levels, values per 100 g of food are required. A conversion factor derived from the proportion of the total lipid present as FAs is required for converting percentages of total FAs to FAs per 100 g of food. Total lipid level was multiplied by a conversion factor of 0.80 to convert it to total fatty acids [13]. (0.80 is a conversion factor to convert total lipid to total fatty acids.) For fatty acids, precision is best limited to 0.1 g/100 g of fatty acids [14], with trace being set at < 0.06 g/100 g total fatty acids.

Statistical analyses

Statistical analyses were carried out to determine mean, standard deviation, coefficient of variation in per cent. Further statistical analysis was carried out using the Chi-square (X^2) method as appropriate, the α value for the X^2 was 0.05 [15].

Results and Discussion

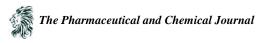
Table1: Levels of crude fat, total fatty acid (g/100g) and total energy (kJ/100g) in *Bridelia ferruginea* stem bark (IR), *Monodora myristica* seed kernel (MD), and *Zingiber officinale Roscoe* (GG) samples

* **						,		. ,	
Parameters	IR	MD	GG	Mean	SD	CV %	X^2	TV	Remark
Crude Fat	1.40	30.5	8.50	13.5	15.2	113	34.2	5.991	S
*Total Fatty acids (g/100g)	1.12	24.4	6.8	10.8	12.1	113	27.4	5.991	S
**Energy(kJ/100g)	41.4	903	252	399	449	113	1012	5.991	S

*crude fat x 0.800,** total fatty acid x 37, SD = standard deviation, CV = coefficient of variation, X^2 = Chi-square, TV= critical table value at α = 0.05

Table 2: Fatty acids (%) composition of *Bridelia ferruginea* stem bark (IR), *Monodora myristica* seed kernel (MD), and *Zingiber officinale Roscoe* (GG) samples

Fatty acids	IR	MD	GG	Mean	SD	CV%	X^2	TV	Remark
C6:0	0.00	0.00	1.72e-6	5.73e-7	9.93e-7	173	0.00	5.991	NS
C8:0	0.00	0.00	2.26	0.753	1.30	173	4.52	5.991	NS
C10:0	0.00	0.00	3.53	1.18	2.04	173	7.06	5.991	S
C12:0	0.00	0.00	9.14	3.05	5.28	173	18.3	5.991	S
C14:0	2.09	0.00	4.25	2.11	2.13	101	4.27	5.991	NS
C16:0	22.4	14.0	21.9	19.4	4.71	24.2	2.29	5.991	NS
C18:0	7.51	2.77	5.16	5.15	2.37	46.0	2.18	5.991	NS
C20:0	0.238	0.225	1.67e-7	0.154	0.134	86.7	0.230	5.991	NS
C22:0	0.220	0.207	1.54e-6	0.142	0.123	86.7	0.210	5.991	NS
C24:0	0.027	0.026	1.92e-7	0.018	0.015	86.6	0.030	5.991	NS
Total SFA	32.5	17.2	46.2	32.0	14.5	45.4	13.2	5.991	S
C14:1(Cis-9)	0.126	0.00	6.24e-7	0.042	0.073	173	0.250	5.991	NS
C16:1(Cis-9)	18.9	0.511	0.051	6.49	10.8	166	35.6	5.991	S
C18:1(Cis-6)	12.5	9.32	11.2	11.0	1.60	14.5	0.460	5.991	NS
C18:1(Cis-9)	18.9	9.36	14.0	14.1	4.77	33.9	3.23	5.991	NS
C20:1 (Cis-11)	0.900	0.849	0.162	0.637	0.412	64.7	0.530	5.991	NS
C22:1(Cis-13)	0.076	0.0712	1.30e-6	0.049	0.043	86.7	0.070	5.991	NS
C24:1(Cis-15)	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-
Total MUFA Cis	51.4	20.1	25.4	32.3	16.7	51.8	17.4	5.991	S



C18:1 (trans-6)	0.086	0.081	8.39e-7	0.056	0.048	86.7	0.084	5.991	NS
C18:1 (trans-9)	0.008	0.0073	5.43e-8	0.005	0.004	86.9	0.080	5.991	NS
C18:1 (trans-11)	0.00	0.00	1.60e-6	5.33e-7	9.24e-7	173	0.00	5.991	NS
Total MUFA trans	0.094	0.088	2.49e-6	0.061	0.053	86.7	0.091	5.991	NS
MUFA Total	51.5	20.2	25.4	32.4	16.8	51.8	17.4	5.991	S
C18:3 (Cis-9,12, 15)	2.32	0.555	2.00	1.63	0.940	57.9	1.09	5.991	NS
C20:2 (Cis-11,14)	0.034	0.032	4.85e-7	0.022	0.019	86.7	0.033	5.991	NS
C20:3 (Cis-11,14,17)	0.146	0.137	1.02e-6	0.094	0.082	86.7	0.142	5.991	NS
C20:5 (Cis-5,8,11,14,17)	0.00	0.00	3.74e-7	1.25e-7	2.16e-7	173	0.00	5.991	NS
C22:6 (<i>Cis</i> -4,7,10,13,16,19)	0.00	0.00	1.37e-7	4.57e-8	7.91e-8	173	0.00	5.991	NS
Total (<i>n</i> -3)	2.50	0.724	2.00	1.74	0.916	52.6	0.964	5.991	NS
C18:2 (Cis-9,12)	28.1	60.6	24.7	37.8	19.8	52.4	20.8	5.991	S
C18: 2 (trans-9,11)	0.100	0.095	7.03e-7	0.065	0.056	86.7	0.098	5.991	NS
C18:3 (Cis-6,9,12)	2.12	0.488	1.73	1.45	0.852	58.9	1.00	5.991	NS
C20:3 (Cis-8,11,14)	0.766	0.097	1.86e-6	0.288	0.417	145	1.21	5.991	NS
C20:4 (Cis-5,8,11,14)	0.159	0.097	2.00e-5	0.085	0.080	93.9	0.150	5.991	NS
C22:2 (Cis-13,16)	0.027	0.026	1.08e-6	0.018	0.015	86.6	0.027	5.991	NS
Total (<i>n</i> -6)	31.3	61.4	26.4	39.7	18.9	47.7	18.1	5.991	S
PUFA Total	33.8	62.1	28.4	41.4	18.1	43.7	15.8	5.991	S

SFA= saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, NS = not significant, S= significant

Table 3: Summary of the quality parameters of fatty acids of *Bridelia ferruginea* stem bark (IR), *Monodora myristica* seed kernel (MD), and *Zingiber officinale Roscoe* (GG) samples

Important parameter	IR	MD	GG	Mean	SD	CV%	X^2	TV	Remark
SFA	32.5	17.2	46.2	32.0	14.5	45.4	13.2	5.991	S
MUFA Cis	51.4	20.1	25.4	32.3	16.7	51.8	17.4	5.991	S
MUFA trans	0.094	0.088	2.49e-6	0.061	0.053	86.7	0.091	5.991	NS
MUFA Total	51.5	20.2	25.4	32.4	16.8	51.8	17.4	5.991	S
n-3 PUFA	2.50	0.724	2.00	1.74	0.916	52.6	0.964	5.991	NS
n-6 PUFA	31.3	61.4	26.4	39.7	18.9	47.7	18.1	5.991	S
Total PUFA	33.8	62.1	28.4	41.4	18.1	43.7	15.8	5.991	S
DUFA Cis	2.50	0.724	2.00	1.74	0.916	52.6	0.964	5.991	NS
DUFA trans	0.100	0.095	7.03e-7	0.065	0.056	86.7	0.098	5.991	NS
DUFA total	2.60	0.819	2.00	1.81	0.906	50.2	0.909	5.991	NS
TUFA Cis	5.35	1.28	3.73	3.45	2.05	59.4	2.44	5.991	NS
TUFA trans	-	-	-	-	-	-	-	-	
TUFA total	5.35	1.28	3.73	3.45	2.05	59.4	2.44	5.991	NS
MUFA/SFA	1.59	1.17	0.55	1.10	0.52	47.3	0.493	5.991	NS
PUFA/SFA	1.04	3.61	0.61	1.75	1.62	92.3	2.99	5.991	NS
n-6/ n -3	12.5	84.8	13.2	36.9	41.6	113	93.7	5.991	S
EPSI	0.656	3.08	1.12	1.62	1.28	79.4	2.04	5.991	NS
LA/ALA	12.1	109	12.4	44.6	56.0	126	141	5.991	S

DUFA= diunsaturated fatty acid, TUFA = triunsaturated fatty acid, EPSI= essential PUFA status index, LA = linoleic acid, ALA = alpha linolenic acid, -= not detected



Table 4: Fatty acids g/100g as food of *Bridelia ferruginea* stem bark (IR), *Monodora myristica* seed kernel (MD), and *Zingiber officinale Roscoe* (GG) samples

Fatty acids	IR	MD	GG	Mean	SD	CV%
C6:0	0.00	0.00	1.17e-7	3.90e-8	6.75e-8	173
C8:0	0.00	0.00	0.154	0.051	0.089	173
C10:0	0.00	0.00	0.240	0.080	0.139	173
C12:0	0.00	0.00	0.622	0.207	0.359	173
C14:0	0.023	0.00	0.289	0.104	0.161	154
C16:0	0.251	3.42	1.49	1.72	1.59	92.8
C18:0	0.084	0.676	0.351	0.370	0.296	80.0
C20:0	0.0027	0.055	1.14e-8	0.019	0.031	161
C22:0	0.0025	0.051	1.05e-7	0.018	0.028	161
C24:0	0.000	0.006	1.31e-8	0.002	3.58e-3	162
Total SFA	0.364	4.20	3.14	2.57	1.98	77.1
C14:1(Cis-9)	0.0014	0.00	4.24e-8	4.70e-4	8.15e-4	173
C16:1(Cis-9)	0.212	0.125	3.44e-3	0.113	0.105	92.3
C18:1(<i>Cis</i> -6)	0.140	2.27	0.762	1.06	1.10	104
C18:1(<i>Cis</i> -9)	0.212	2.28	0.952	1.15	1.05	91.4
C20:1 (Cis-11)	0.010	0.21	1.10e-2	0.076	0.114	149
C22:1(Cis-13)	0.001	0.017	8.84e-8	6.07e-3	9.79e-3	161
C24:1(<i>Cis</i> -15)	0.00	0.00	0.00	0.00	0.00	0.00
Total MUFA Cis	0.576	4.91	1.73	2.40	2.24	93.3
C18:1 (trans-6)	0.001	0.020	5.71e-8	6.91e-3	0.011	161
C18:1 (trans-9)	0.00	1.78e-3	3.69e-9	6.24e-4	1.00e-3	161
C18:1 (trans-11)	0.00	0.00	1.09e-7	3.63e-8	6.28e-8	173
Total MUFA trans	0.001	0.022	1.70e-7	7.53e-3	0.012	161
MUFA Total	0.577	4.93	1.73	2.41	2.25	93.5
C18:3 (Cis-9,12, 15)	0.026	0.135	0.136	0.099	0.063	63.9
C20:2 (Cis-11,14)	0.00	0.008	3.30e-8	2.71e-3	4.37e-3	161
C20:3 (Cis-11,14,17)	0.002	0.033	6.94e-8	0.012	0.019	161
C20:5 (Cis-5,8,11,14,17)	0.00	0.00	2.54e-8	8.48e-9	1.47e-8	173
C22:6 (<i>Cis</i> -4,7,10,13,16,19)	0.000	0.00	9.32e-9	3.11e-9	5.38e-9	173
Total (<i>n</i> -3)	0.028	0.18	0.136	0.11	0.077	68
C18:2 (Cis-9,12)	0.315	14.8	1.68	5.59	7.99	143
C18: 2 (trans-9,11)	0.001	0.023	4.78e-8	8.10e-3	0.013	161
C18:3 (Cis-6,9,12)	0.024	0.119	0.118	0.087	0.055	62.9
C20:3 (Cis-8,11,14)	0.009	0.024	1.26e-7	0.011	0.012	111
C20:4 (Cis-5,8,11,14)	0.002	0.024	1.36e-6	8.48e-3	0.013	155
C22:2 (Cis-13,16)	0.000	0.006	7.34e-8	2.22e-3	3.58e-3	162
Total (<i>n</i> -6)	0.350	15.0	1.80	5.71	8.06	141
PUFA Total	0.378	15.2	1.93	5.82	8.12	139

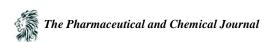
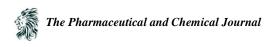


Table 5: Energy (kJ/100g) contributions from the fatty acids compositions of *Bridelia ferruginea* stem bark (IR), *Monodora myristica* seed kernel (MD), and *Zingiber officinale Roscoe* (GG) samples

Fatty acids	IR	MD	GG	Mean	SD	CV%
C6:0	0.00	0.00	4.33e-6	1.40e-6	2.50e-6	173
C8:0	0.00	0.00	5.69	1.90	3.28	173
C10:0	0.00	0.00	8.88	2.96	5.13	173
C12:0	0.00	0.00	23.0	7.67	13.3	173
C14:0	0.866	0.00	10.7	3.85	5.94	154
C16:0	9.28	126	55.1	63.6	59.0	92.8
C18:0	3.11	25.0	13.0	13.7	11.0	80.0
C20:0	0.099	2.03	4.20e-7	0.710	1.15	161
C22:0	0.091	1.87	3.87e-6	0.653	1.05	161
C24:0	0.011	0.23	4.83e-7	0.082	0.13	162
Total SFA	13.5	156	116	95.1	73.4	77.1
C14:1(Cis-9)	0.052	0.00	1.57e-6	0.017	0.03	173
C16:1(<i>Cis</i> -9)	7.83	4.61	0.127	4.19	3.87	92.3
C18:1(<i>Cis</i> -6)	5.18	84.1	28.2	39.2	40.6	104
C18:1(<i>Cis</i> -9)	7.83	84.5	35.2	42.5	38.9	91.4
C20:1 (Cis-11)	0.373	7.66	0.41	2.82	4.20	149
C22:1(Cis-13)	0.031	0.643	0.00	0.225	0.362	161
C24:1(Cis-15)	0.00	0.00	0.00	0.00	0.00	0.00
Total MUFA Cis	21.3	182	63.9	88.9	83.0	93.3
C18:1 (trans-6)	0.036	0.731	2.11e-6	0.256	0.412	161
C18:1 (trans-9)	0.00	6.59e-2	1.37e-7	0.023	0.037	161
C18:1 (trans-11)	0.00	0.00	0.00	1.30e-6	0.000	173
Total MUFA trans	0.039	0.797	6.27e-6	0.279	0.449	161
MUFA Total	21.3	182	63.9	89.2	83.4	93.5
C18:3 (Cis-9,12, 15)	0.961	5.01	5.03	3.67	2.34	63.9
C20:2 (Cis-11,14)	0.014	0.287	1.22e-6	0.100	0.162	161
C20:3 (Cis-11,14,17)	0.061	1.24	2.57e-6	0.432	0.697	161
C20:5 (Cis-5,8,11,14,17)	0.00	0.00	9.41e-7	3.10e-7	5.43e-7	173
C22:6 (Cis-4,7,10,13,16,19)	0.00	0.00	3.45e-7	1.10e-7	1.99e-7	173
Total (<i>n</i> -3)	1.04	6.53	5.03	4.201	2.84	68
C18:2 (Cis-9,12)	11.6	547	62.1	207	296	143
C18: 2 (trans-9,11)	0.041	0.86	1.77e-6	0.300	0.484	161
C18:3 (Cis-6,9,12)	0.879	4.41	4.35	3.21	2.02	62.9
C20:3 (Cis-8,11,14)	0.317	0.876	4.68e-6	0.398	0.443	111
C20:4 (Cis-5,8,11,14)	0.066	0.876	5.03e-5	0.314	0.488	155
C22:2 (Cis-13,16)	0.011	0.235	2.72e-6	0.082	0.132	162
Total (n-6)	13.0	554	66.5	211	298	141
PUFA Total	14.0	561	71.5	215	301	139



41.7

76.8

64.0

5.991

S

Total

(MD), and Zingiber officinale Roscoe (GG) samples									
Phospholipids	IR	MD	GG	Mean	SD	CV%	X^2	TV	Remark
Phosphatidylethanolamine	16.6	24.4	7.36	16.1	8.53	52.9	9.03	5.991	S
Phosphatidylcholine	79.8	43.6	49.5	57.6	19.4	33.7	13.1	5.991	S
Phosphatidylserine	0.910	13.2	99.9	38.0	54.0	142	153	5.991	S
Lysophosphatidylcholine	5.91	5.19	3.52	4.87	1.23	25.2	0.617	5.991	NS
Phosphatidylinositol	6.93	177	18.5	67.5	95.0	141	268	5.991	S

Table 6: Levels (mg/100g) of phospholipids in *Bridelia ferruginea* stem bark (IR), *Monodora myristica* seed kernel (MD), and *Zingiber officinale Roscoe* (GG) samples

Table 7: Levels (mg/100g) of phytosterols in *Bridelia ferruginea* stem bark (IR), *Monodora myristica* seed kernel (MD), and *Zingiber officinale Roscoe* (GG) samples

184

179

263

110

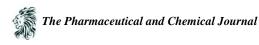
Phytosterols	IR	MD	GG	Mean	SD	CV%	X^2	TV	Remark
Cholesterol	8.54e-5	5.27e-4	2.48e-7	2.04e-4	2.83e-4	138	0.00	5.991	NS
Cholestanol	2.28e-3	2.28e-3	2.02e-3	2.19e-3	1.50e-4	6.8	0.00	5.991	NS
Ergosterol	1.61e-3	1.61e-3	1.14e-3	1.45e-3	2.71e-4	18.7	0.00	5.991	NS
Campesterol	7.28	17.9	8.66	11.3	5.77	51.2	5.91	5.991	NS
Stigmasterol	1.24	10.5	9.55	7.10	5.09	71.8	7.31	5.991	S
5- Avenasterol	0.821	5.29	8.95	5.02	4.07	81.1	6.60	5.991	S
Sitosterol	28.2	68.3	69.0	55.2	23.4	42.3	19.8	5.991	S
Total	37.5	102	96.2	78.6	35.7	45.4	32.4	5.991	S

Crude fat, total fatty acids (g/100g) on dry weight basis and energy (kJ/100g) are presented in Table 1. Levels of the crude fat in the samples ranged between 1.40 and 30.5 with the highest value occurring in *Monodora myristica* seeds kernel flour, the calculated total fatty acid levels ranged from 1.12 to 24.4 whereas the energy values have the values in the samples between 41.4 and 903. The CV % values of 113 showed that the levels of these parameters were widely varied giving a trend in the order of *Monodora myristica* seeds kernel > *Bridelia ferruginea* stem bark > *Zingiber officnale Roscoe* (Ajo).

The crude fat results in the present report were better than the values (g/100g) reported for raw and processed pigeon pea varieties (0.33 - 1.1) [16], millet (1.10) and rice (0.63) [17], black gram mash varieties (0.97 - 1.42) [18], some vegetables commonly consumed in Ekiti State [19] but comparably lower than the values reported for dika nut kernel flour [20], raw and processed soybean flour [21]. Except *Bridelia ferruginea* stem bark sample which had the least fat content, both *Monodora myristica* seeds kernel and *Zingiber officnale Roscoe* (Ajo) flours could be regarded as major sources of dietary fat. The total fatty acid (TFA) profiles showed that *Monodora myristica* seeds kernel flour had the highest value (24.4g/100g) and the lowest in *B. ferruginea* stem bark flour with a value of 1.12g/100g.

Crude fat, total fatty acids and energy contents (kJ/100g) were significantly different on their groups when subjected to X^2 (Chi-square) analysis at $\alpha_{=0.05}$. The significant difference would have been due to the highest value of those parameters in *Monodora myristica* seed kernel flour.

In Table 2, fatty acid profiles of the samples in %total fatty acids were shown. The following fatty acids (FAs) recorded 0.00% value in *B. ferruginea* stem bark and *Monodora myristica* seeds kernel flours C6:0, C8:0, C10:0 and C12:0 whereas the values for these fatty acid in *Zingiber officnale Roscoe* (Ajo) ranged between 1.72e⁻⁶ - 9.14%. In all the samples, the most concentrated saturated fatty acid (SFA) come from C16:0 having a range of 14.0-22.4% with coefficient of variation % value of 24.2 whereas total SFA range was 17.2 - 46.2% and a CV% value of 45.4. This meant that the SFA values were generally widely varied by the fairly high CV%. The total mono-saturated fatty



acid cis-configuration (MUFA*cis*) in the samples was within the range of 20.1 and 51.4% and a CV% value of 51.8. These values were apparently higher than the SFAs. Meanwhile the trans-version of the MUFA values was generally low between 2.49e-6 - 0.094%. These values were comparably close to the levels reported for citrus seeds [22]. Among the poly unsaturated fatty acids (PUFAs) C18:2 (cis-9, 12) had significant high levels in all the samples, the range being 247 - 60.6% and a CV% value of 52.4. These values were comparably higher than those reported for various types of chilles consumed in Nigeria [23] with respect to the total PUFAs in the samples, C18:2 (*cis*-9, 12) constitutes the highest percentages (*B. ferruginea* stem bark, 83.1, *Monodora myristica*, 97.6, and *Zingiber officnale Roscoe* (Ajo), 87.0) meaning that the major contributions to the PUFAs total were from C18:2(*cis*-9,12).

Also from Table 2, the chi-square (X^2) analysis showed significant differences between the following parameters of fatty acids. Total SFA, C16:1(cis-9), MUFA, Total MUFA, C18:2(cis-9,12), n-6 and Total PUFA at α =0.05.

Summary of the calculated quality parameters of fatty acids from Table 2 were shown in Table 3. These parameters are essential in the prediction of the nutritional qualities of the three locally available species used in this research work as contained in their lipid compositions.

From the Table 3 results, Chi-square (X^2) analysis showed that seven parameters: SFA, MUFA cis, MUFA total, n-6, PUFA total, n-6/n-3 and LA/ALA had their critical (table) values at $\alpha_{=0.05}$ less than the calculated values (samples) thereby Hayes [24] reported that the best dietary fat would contain an idea balance (7:1) of n-6/ n^{-3} (i.e. Linoleic /linolenic acids. This balance is not available in partially hydrogenated margarines, in which most of the n-3 linolenic acid has been destroyed by processing, and is also unlike most vegetable oils that contain only a small amount of this important fatty acid. n-6/n-3 and LA/ALA ratios in the present report (12.5:1 – 84.4:1 and 12.1:1-109:1 respectively) were shown to be highly deviated from 7:1 as recommended. The reason for these could be due to high level of n-6 and LA (26.4-61.4 and 24.7-60.6% respectively) and very low levels of n-3 and ALA (0.724-2.50 and 0.555-2.32% respectively) in these three samples.

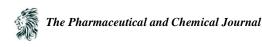
The detrimental effect of dietary fat is determined by the PUFA/SFA ratio. The higher the PUFA/SFA ratio, the more nutritionally useful is the oil. The ratio obtained for the samples in the present reports ranged between 0.61 and 3.61. These ratios were comparably lower than those reported for citrus seed oils [22]. Honatra, [25] had stressed the fact that the proportions of the total energy supplied by SFA and PUFA fats determined the seventy of atherosclerosis. In the present reported, these proportions were positive towards PUFA far more than the SFA.

The MUFA/SFA levels in the samples ranged from 0.55-1.59 which was less than in the PUFA/SFA levels. Adeyeye and Adesina [22] reported that the relative proportions of MUFA/SFA is an important aspect of phospholipids compositions and change to this ratio have been claimed to have effects on such disease states as cardiovascular diseases, obesity, diabetes, cancer and neuropathological condition.

Furthermore, cytoprotective actions in pancreatic β -cells as well as exhibition of desirable physical properties for membrane lipids have been attributed to MUFA/SFA. They are also known to be relatively resistant to oxidative degradation.

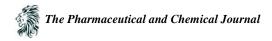
The essential PUFA status index (EPSI), a ratio which includes the sum of all n-3, n-6 and sum of all n-7 and n-9 FAs: is an indicator of essential PUFA status. This ratio indicates how nutritionally good the oil is considering the PUFA with reference to MUFA in the sample in the sample. The higher the EPSI, the better is the oil. The EPSI values in the samples ranged from 0.656 to 3.08. The Chi-square (X^2) analysis at $\alpha_{=0.05}$ showed that significant differences existed between the following parameters: SFA, MUFA cis, MUFA total, n-6, PUFA total, n-6/n-3 and LA/ALA.

In Table 4, the fatty acids calculated as food lipids (g/100g) were shown. Groups of fatty acid of significant contribution in lipid food composition were (g/100g): SFA (B. ferruginea stem bark, 0.364, Monodora myristica seed kernel, 420, and Zingiber officnale Roscoe (Ajo), 3.14), total MUFA (B. ferruginea stem bark, 0.576, Monodora myristica seed kernel, 4.91 and Zingiber officnale Roscoe (Ajo), 1.73), C18:2(cis-9,12) (B. ferruginea stem bark, 0.315, Monodora myristica seed kernel, 14.8, Zingiber officnale Roscoe (Ajo), 1.68) and n-6 (B. ferruginea stem bark, 0.350, Monodora myristica seed kernel, 15.0 and Zingiber officnale Roscoe (Ajo), 1.80). to be able to calculate the energy contribution from each fatty acids, the information obtained from the food values (g/100g) is necessary. As expected therefore, the energy contribution shown in Table 5 are as follows, considering



the ones with major contributions (kJ/100g) SFA (*B. ferruginea* stem bark,13.5, *Monodora myristica* seed kernel,156, *Zingiber officnale Roscoe* (Ajo),116), MUFA total (*B. ferruginea* stem bark,21.3, *Monodora myristica* seed kernel,182, *Zingiber officnale Roscoe* (Ajo),63.9), C18:2(cis-9,12), (*B. ferruginea* stem bark,11.6, *Monodora myristica* seed kernel,547, *Zingiber officnale Roscoe* (Ajo),62.1) and *n*-6(*B. ferruginea* stem bark,13.0, *Monodora myristica* seed kernel,554, *Zingiber officnale Roscoe* (Ajo),66.5). The highest contribution came from *Monodora myristica* seed kernel among all the samples.

Table 6 shows the levels (mg/100g) of the various phospholipids in the samples. Phospholipids are not essential nutrients; they are just another lipid and, as such, contribute 9 kcalories per gram. Cephalin (phosphatidylethanolamine, PE) was the second largest concentrated entity in muscle and in skin. PE is found in all living cells, although in human physiology it is found particularly in nervous tissue such as the white matter of brain, nerves, neutral tissue and in spinal cord [26]. Phosphatidylserine (Ptd-L-Ser or PS) is a phospholipid usually kept on the inner-leaflet, the cytosolic side, of cell membranes by an enzyme called flippase. When a cell undergoes apoptotic cell death, PS is no longer restricted to the cytosolic part of the membrane, but becomes exposed on the surface of the cell. PS has been demonstrated to speed up recovery, prevent muscle soreness, improve well-being, and might possess ergogenic properties in athletes involved in cycling, weight training and endurance running. PS supplementation promotes a desirable hormonal balance for athletes and might attenuate the physiological deterioration that accompanies overtraining and/or overstretching [27]. In recent studies, PS has been shown to enhance mood in a cohort of young people during mental stress and to improve accuracy during tee-off by increasing the stress resistance of golfers [28]. The US Food and Drug Administration (USFDA) had stated that consumption of PS may reduce the risk of dementia in the elderly and may also reduce the risk of cognitive dysfunction in the elderly. PS can be found in meat, but most abundant in the brain and innards such as liver and kidney. The present results recorded 0.910 mg/100 g in the Bridelia ferruginea stem bark sample, 13.2 mg/100 g in the Monodora myristica seed kernel and 99.9 mg/100g in Zingiber officinale Roscoe (Ajo) which were higher than the values in Aframomum melegueta and Xylopia aethiopica [29], grape, orange and tangerine seed oli [22] and European pilchard (sardine) of 16.0 mg/100 g [28]. Phosphatidylcholine (lecithin) is usually the most abundant phospholipids in animal and plants, often amounting to almost 50 % of the total, and as such it is the key building block of membrane bilayers. This observation is true for lecithin value in Bridelia ferruginea stem bark (79.8 mg/100 g or 72.5 %), Monodora myristica seed kernel (43.6 mg/100 g or 16.6 %) and Zingiber officinale Roscoe (Ajo) (49.5 mg/100g or 27.7 %). Lecithin is also the principal phospholipids circulating in plasma, where it is an integral component of the lipoproteins, especially the HDL. It is a neutral or zwitterionic phospholipid over a pH range from strongly acid to strongly alkaline; it is used as an emulsifier in the food industry. Certain enzymes in snake venom can cause the hydrolysis of the unsaturated fatty acids on the β -carbon atom of phospholipids, resulting in the production of compounds known as lysolecithins (and lysocephalins), substances which have strong haemolytic action. Death may result if the haemolysis in the victim of a snake bite is extensive enough [30]. Large doses of lecithin may cause gastrointestinal upsets, sweating, salivation and loss of appetite [31]. Phosphatidylinositol (PtdIns, PI) is a negatively charged phospholipid and a minor component in the cytosolic side of eukaryotic cell membranes. The inositol can be phosphorylated to form phosphatidylinositol phosphate (PIP), phosphatidylinositol bisphosphate (PIP₂) and phosphstidylinositol trisphosphate (PIP₃). PIP, PIP₂, and PIP₃ are collectively called phosphoinositides. Phosphoinositides play important roles in lipid signaling, cell signaling and membrane trafficking [32]. PI was of major concentration in *Monodora myristica* seed kernel sample (177 mg/100g or 65.8 %) compared to Bridelia ferruginea stem bark and Zingiber officinale Roscoe (Ajo) samples which are of low concentrations. Partial hydrolysis of lecithin with removal of only one fatty acid yields a lysophosphatidylcholine [32]. An example of alterations in enzymatic activity related to association of a membrane -bound protein with lipid is that of phenylalanine hydroxylase, which catalyzes the conversion of phenylalanine to tyrosine. The activity of these enzymes, which is attached to the endoplasmic reticulum, is enhanced fifty fold in the presence of lysophosphstidylcholine, with which it is probably complexed in the hepatic cell [32]. Lysophosphatidylcholine was of low levels in all the samples (Bridelia ferruginea stem bark, 5.91 mg/100g 5.37 %

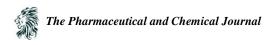


or; *Monodora myristica* seed kernel, 5.19 mg/100g or 1.97 % and *Zingiber officinale Roscoe*, 3.52 mg/100g or 1.97 %), these values were comparably lower than vales reported for various types of chilies [23].

Statistical analysis (Chi-square, X^2) of the results in Table 6 showed that there were significant differences between all the results except lysophosphatidylcholine values. This was basically due to the ways in which these phospholipids were distributed in the various samples, for instance, the most concentrated phopspholipid in the *Bridelia ferruginea* stem bark sample was phosphatidylcholine, in *Monodora myristica* seed kernel was phosphatidylinositol whereas Phosphatidylserine was of the highest concentration in *Zingiber offficinale Roscoe* (Ajo).

The sterol levels were shown in Table 7. The values (mg/100 g) in the cholesterol, cholestanol, ergosterol, campesterol, stigmasterol, 5-Avenasterol and sitosterol range were in all the samples as: 8.54e-5 - 28.2, in Bridelia ferruginea stem bark, 5.27e-4 - 68.3 in Monodora myristica seed kernel and 2.48e-7 - 69.0 in Zingiber officinale Roscoe (Ajo). As expected, the three samples had an appreciable amount of sitosterol and so could contribute meaningfully in human nutrition. The good aspects of cholesterol included being present in mammalian cell membranes where it is required to establish proper membrane permeability and fluidity, a precursor molecule for the biosynthesis of bile acids, steroid hormones and several fat soluble vitamins. Cholesterol does exert one negative influence in the body, however. On its way into cells from the blood stream, some cholesterol forms deposits in the artery walls. These deposits lead to atherosclerosis, a disease that causes heart attacks and strokes. Complex lipids are bonded to other types of molecules. Because lipids are mostly insoluble in water, the movement of lipids from organ to organ through the bloodstream is facilitated by plasma lipoproteins. The four major classes of plasma proteins are: chylomicrons [density = < 0.95 g/ml], function: carry triglycerides from intestines to other tissues; VLDL (very low density lipoproteins) [density = 0.95-1.019 g/ml], function: carry triglycerides from liver; LDL (low density lipoproteins) [density = 1.019-1.063 g/ml], function: carry cholesterol to peripheral tissues; HDL (high density lipoprotein) [density =1.063-1.210 g/ml], function: carry cholesterol from peripheral tissues to liver. Dietary patterns can also affect the metabolism of cholesterol. However, diet low in saturated fat, trans fat and cholesterol encourage the uptake of LDL by the liver, thereby removing LDL from the blood stream and decreasing the ability of scavenger cells to form atherosclerotic plagues in the blood vessels. Likewise, diets high in saturated fat, trans fat and cholesterol reduce the uptake of LDL by the liver, increasing cholesterol in the blood and the risk for cardiovascular disease [31]. The total dietary fats and oils range from 0.01-2 % [33]; the present levels were 1.40 % in the Brideelia ferruginea stem bark, 30.5 % in Monodora myristica seed kernel and 8.50 % in the Zingiber officinale Roscoe (Ajo) which were within the literature values. Stigmasterol shared second and third positions in Monodora myristica seed kernel and Bridelia ferruginea stem bark samples with respective values of 10.5 and 9.55 mg/100 g. Stigmasterol is used as a precursor in the manufacture of synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens and corticoids. Research has indicated that stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast and colon cancers. Studies have also indicated that a diet high in phytosterols may inhabit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption. Studies with laboratory animals fed stigmasterol found that both cholesterol and sitosterol absorption decreased 23 % and 30 % respectively over a 6 week period. Stigmasterol is also known as Wulzen anti-stiffness factor. Cholesterol enters the intestinal tract by excretion across the intestinal mucosa as well as via the bile. In the rumen of the gut a portion is reduced microbially to coprostanol and cholestanol and thereby is excluded from reabsorption. These two stanols, together with cholesterols, constitute the bulk of the fecal sterols. Certain of these transformations, e.g., from cholestenone to cholestanol, also occur in the liver [32].

The Chi-square (X^2) analysis showed that there was no significant difference between the values for the following phytosterols: cholesterol, cholesterol, Ergosterol and campesterol whereas significant differences existed between the results of the following among the samples: Stigmasterol, 5-Avenasterol, sitosterol as well as the total phytosterols.

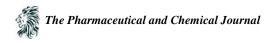


Conclusion

The findings of this study showed that the samples contained unequal distribution of all the parameters determined. Both samples were high in *n*-6 fatty acids but low in *n*-3 fatty acids. The three samples had unsaturated acids as the predominant fatty acids. Significant difference occurred among some quality parameters of the fatty acid levels, phospholipids (except lysophosphatidylcholine) and few of the phytosterols. The samples would serve as average source of lecithin (*Bridelia ferruginea* stem bark), phophatidylinositol (*Monodora myristica* seed kernel) and Phosphatidylserine (*Zingiber officinale Roscoe* (Ajo) but were generally low in sterols except sitosterols particularly *Monodora myristica* seed kernel and *Zingiber officinale Roscoe* (Ajo). Quality assurances of the determinations were highly satisfactory. The various levels of parameters analysed in the samples: total fatty acids, phospholipids and phytosterols, hence their consumption, as food sources either as spice or soup ingredients may not result in the consumers consuming fats above the recommended healthy allowances.

References

- 1. Leslie, B. Spices & Herbs. Penguin Group (USA) Inc. (2007). 375 Hudson Street, New York, New York 10014, USA.
- 2. Peter, K.V. (2001). *Handbook of herbs and spices*. CRC Press LLC Corporate Blvd, NW Boca Raton FL 33431, USA.
- 3. Ampofo, O. (1979). *The practice of phytotheraphy in Ghana*. In: Sofowora E. A. (Ed.) *African Medicinal Plants*. University of Ife Press, Ile-Ife, p 67.
- Njamen, D., Benedicta, N. N., Tsala, E., Fomum, Z. T., Mbanya, J. C. & Ngufor, G. F. (2013). Effect of Bridelia Ferruginea (Euphobiaceae) leaf extract on sucrose –induced glucose intolerance in rats. Tropical Journal of Pharmaceutical Research, 11(5): 759 – 765. http://dx.doi.org/10.4314/tjpr.v11i5.9
- 5. Iwu. M. M. (1980). Anti-diabetic properties of Bridelia Ferruginea leaves. Planta Medica, 39: 247.
- 6. Tella, E., Njamen, D., Djoulde, D. R., Tatsadejeu, L., Tantoh, D., Mbafor, J. T. & Fomum, Z. T. (2002). Anti-microbial activity of *Bridelia Ferruginea* leaves extracts. *Fitoterapia*, 73:343 345. http://dx.doi.org/10.1016/s0367-326x(02)00084-9
- 7. Adesina, A. J, & Akomolafe, S. F.(2014). Nutritional and Anti-Nutritional Composition of *Bridelia Ferruginea* Benth (*Euphorbiaceae*) Stem Bark Sample. *International Journal of Scientific Research in Knowledge*. 2(2):92-104. http://dx.doi.org/10.12983/ijsrk-2014-p0092-0104
- 8. Eggeling, W. J. (2012). *The indigenous trees of the Uganda protectorate (Revised and enlarged by Ivan R. Dale)*. Government printer, Entebbe, Uganda, Crown Agents for the Colonies, London. Pp, xiii and 491.
- 9. Adesina, A. J, Gbolagade, A. Y., Olatoye, R. A. & Adegalu, A. A. (2015). Nutritional and anti-nutritional composition of calabash nutmeg (*Monodora Myrystica* Gaertn.) Dunal seed kernel. *Journal of Chemical, Biological and Physical Sciences*. 5(4): 3652 3667.
- 10. European Medicines Agency (2012). Assessment report on Zingiber officinale Roscoe, rhizome. EMA/HMPC/577856/2010, Committee on Herbal Medicinal Products (HMPC).
- 11. A.O.A.C (2005). *Official methods of analysis*, 18th edition, Association of Official Analytical Chemists, Washington DC, USA, 969.33, 996.06 and 970.51.
- 12. Raheja, R. K, Kaur, C., Singh, A. & Bhatia, I. S. (1973). New colorimetric method for the quantitative estimation of phospholipids without digestion. *J. Lipid Res.*, 14: 695-697.
- 13. Anderson, B. A. (1976). Comprehensive evaluation of fatty acids in foods, VII, Pork products. *J. Amer. Diet Assoc.*, 69: 44-49.
- 14. Greenfield, H. & Southgate, D. A. T. (2003). *Food composition data: production, management and use*, 2nd ed. Rome, FAO, 163-170.
- 15. Kothari, C. R. & Gaurav, G. (2014). *Research methodology. Methods and Techniques 3rd Edition*. New Age International (P) Limited, Publishers, 7/30A, Daryagani, New Delhi, India.



- Fasoyiro, S. B., Ajibade, S. R., Saka, J. O., Ashaye, O. A., Obatolu, V. A., Farinde, E. O. & Afolabi, O. O. (2005). Physical characteristics and effects of processing methods on pigeon pea varieties. *Journal of Food Agriculture and Environmental*. 3(3 & 4):59 61.
- 17. Adeyeye, A., & Ajewole, K. (1992). Chemical composition and fatty acid profiles of cereals in Nigeria. *Food Chemistry*. 44:41-44. http://dx.doi.org/10.1016/0308-8146(92)90255-z
- 18. Yasmeen, A., Yaseen, T., Nasreen, Z., Ali, S., Usman, S. & Nasir, S. (2015). Development of health foods from black gram (mash). *Sky Journal of Agricultural Research*, 4(3):42-46.
- 19. Adesina, A. J. (2013). Proximate, Minerals and Anti-nutritional Composition of Three Vegetables Commonly Consumed in Ekiti State, Nigeria. *International Journal of Pharmaceutical and Chemical Sciences*, 2 (3):1631-1638.
- 20. Adeyeye, E. I. (2013). Proximate, mineral and anti-nutrient composition of dika nut (*Irvingia gabonensis*) kernel. *Elixir Food Science*, 38: 14902 14906.
- 21. Ramadan, E. A. (2012). Effect of processing and cooking methods on the chemical composition, sugars and phytic acid of Soybeans. *Food and Public Health*. 2(1):11 15. http://dx.doi.org/10.5923/j.fph.20120201.03
- 22. Adeyeye, E. I. & Adesina, A. J. (2015). Citrus seeds oils as sources of quality edible oils. *Int. J. Curr. Microbiol. App. Sci.*, 4(5): 537-554.
- 23. Adeyeye, E. I., Jimoh, O. A. & Adesina, A. J. (2013). Lipid Composition of the Seeds of Three Types of Chillies Consumed in Nigeria. *Open journal of analytical chemistry research*. 1(3):69-79. http://dx.doi.org/10.12966/ojacr.11.04.2013
- 24. Hayes, K. C. (2002). Dietary fat and heart health: in search of the ideal fat. Asia Pacific *Journal of Nutrition*. 11(Suppl.): S394-S400. http://dx.doi.org/10.1046/j.1440-6047.11.s.7.13.x
- 25. Hornstra, G. (1973). Dietary fats and arterial thrombosis. *Heamostasis*, 2: 21-52. http://dx.doi.org/10.1159/000214006
- 26. Pepeu, G. A. (1996). Review of phosphatidlyserine pharmacological and clinical effects. Is Phosphatidylserine a drug for the ageing brain? *Pharmacol. Research.* 33:73-80. http://dx.doi.org/10.1006/phrs.1996.0013
- 27. Starks, M. A, Starks, S. L., Kingsley, M., Purpura, M. & Jeger, R. (2008). The effects of phosphatidylserine on endocrine response to moderate intensity exercise. *Journal of the International Society of Sports Nutrition*. 5: 11. http://dx.doi.org/10.1186/1550-2783-5-11
- 28. Haines, T. H. (2001). Do sterols reduce proton and sodium leaks through lipid bilayers? *Progress in Lipid Research*, 40(4): 299-324. http://dx.doi.org/10.1016/s0163-7827(01)00009-1
- 29. Adeyeye, E. I., Adesina, A. J. & Fagbohun E. D. (2014). Lipid Composition of *Afromomum melegueta*, *Zingiber officinale*, *Afromomum melegueta* and *Xylopic aethiopica*. *BioMed research* (Food & Nutrition Research). 1(1):1-16.
- 30. Ihekoronye, A. I. & Ngoddy, P. O. (1985). *Integrated Food Science and Technology for the Tropics*. Macmillan Publishers Ltd, London.
- 31. Whitney, E. N., Cataldo, C.B. & Rolfes, S. R. (1994). *Understanding normal and clinical nutrition*. West Publishing Company, New York.
- 32. White, A., Handler, P. & Smith, E. L. (1973). *Principles of Biochemistry* (5th edition) McGraw-Hill Kogakusha Ltd, Tokyo.
- 33. Itoh, T., Tamura, T. & Matsumoto, T. (1973). Sterol composition of 19 vegetable oils. *Journal of American Oil Chemists' Society*, 50: 122-125. http://dx.doi.org/10.1007/bf02633564