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BIOCHEMICAL STUDIES ON NIGERIAN *MONODORA TENUIFOLIA* SEED

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ABSTRACT

The nutritive constituents of the seeds of *Monodora tenuifolia* were analyzed to augment the available information on *Monodora tenuifolia* research. Blood glucose and lipid profile were investigated on the flavonoid rich fraction of *M. tenuifolia* in rats. The composition (gkg^{-1}) of alkaloids, cyanogenic glycosides, tannins and flavonoids were 13.3±0.1, 21.2×10^{-2}±0.6, 1.3±0.1, 1.7±0.1 and 11.7±1.1 respectively. The proximate composition (gkg^{-1}) of *M. tenuifolia* seed were crude fibre (262.3±1.2), crude protein (82.6±1.0), crude fat (349.9±1.9), ash (49.9±0.6), moisture (190.0±0.00) and carbohydrate (65.5±4.7). Analysis of the minerals content (gkg^{-1}) yielded calcium (864.0±29.38), sodium (2752.0±140.35), iron (3.34±0.06), zinc (5.26±0.08), potassium (326.4±13.06), magnesium (342.9±13.71) and phosphorus (9.52±0.17), while vitamin analysis yielded vitamin A (10.05±0.17 iu/100 g), C (56.40±0.14 gkg^{-1}) and E (11.71±0.87 iu /100 g), thiamine (0.11±0.01 gkg^{-1}), niacin (0.46±0.32 gkg^{-1}) and riboflavin (0.04±0.01 gkg^{-1}). The results of amino acid analysis showed the total amino acid of *M. tenuifolia* seed was 71.78 of crude protein. The total essential amino acid with Histidine was calculated to be 29.24 of the crude protein. The antinutrient analysis of *M. tenuifolia* shows it contained total phenol (0.8±0.0 gkg^{-1}), oxalates (4.09±1.17 gkg^{-1}), phytates (0.012±0.42 gkg^{-1}) and trypsin inhibitor (0.230±0.42 iu/g). The main fatty acids of the seed oil are linoleic acid (401.7 g kg^{-1}), oleic acid (346.1 g kg^{-1}) and palmitic acid (122.61 g kg^{-1}). The LD_{50} of the flavonoid-rich fraction was found to be above 5000 mg kg^{-1} b.w. After the day 14 study, biochemical markers such as triacylglycerol, very low density lipoprotein increased significantly ($p<0.05$) compared with the control while high density lipoprotein decreased significantly ($p<0.05$). After the dat 28 study, no significant ($p>0.05$) effect was observed on the blood glucose and lipid profile of wistar albino rats compared with the control. The result shows that *M. tenuifolia* seed is rich in important nutrients, has edible oil and has no deleterious effect on blood glucose and lipid profile of albino rats.

Keywords: *Monodora Tenuifolia*, Spice, Nutritive Constituents, Blood Glucose, Lipid Profile, Rats

1. INTRODUCTION

Monodora is a genus of the plant in the family Annonaceae. It contains approximately 35 species, distributed throughout tropical Africa. Two of the species, *Monodora myristica* and *Monodora tenuifolia* are widely used as spices. There are several scientific literatures available on *Monodora* species (Njoku *et al*., 2012; Ekeanyanwu *et al*., 2010). *Monodora tenuifolia* is a plant with a rich ethnobotanical history. In traditional medicine practice, it is widely used to relieve tooth ache, dysentery, diarrhoea, dermatitis, head ache and as vermifuge (Ezenwali *et al*., 2010). They also have good antioxidant activity and can be important in the management and treatment of stress induced diseases (Njoku *et al*., 2012). The seeds are aromatic and used as an ingredient in Trado-medicines in Southern Nigeria. In food, they are used as spices and condiments and flavour enhancers (Ezenwali *et al*., 2010). When roasted, the ground seeds are rubbed on the skin for skin diseases (Njoku *et al*., 2012).

Spices are used in Ayurveda and thought by some to have medicinal properties. *Monodora tenuifolia* is a

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spice that has been shown to possess potent antioxidant activity (Njoku *et al*., 2012). Most of the spices used for dietary, culinary and medicinal purposes are relatively safe, if the dose is taken within the limits of their required use in food. Nevertheless, when the dose is beyond the nutritional or therapeutic use, the safety of the spice requires a thorough evaluation on their deleterious effect. The deleterious effects of spices used in food and traditional medicine are generally not mentioned in the ancient literature, deliberately or inadvertently. However, recent researchers have exposed some of the deleterious effects of the plant derived food and medicine. The present study analysed the nutritive constituents of *Monodora tenuifolia* seeds as well as the effect of the flavonoid rich fraction on blood glucose and lipid profile of wistar rats.

2. MATERIALS AND METHODS

2.1. Plant Materials

Fresh seeds of *Monodora tenuifolia* (**Fig. 1**) were obtained between the month of May and June, 2012 from fruits harvested from the plant growing within the University of Nigeria Nsukka, Enugu State. The fruits and hence the seeds were authenticated by a botanist in the Department of Botany, University of Nigeria through comparison with a voucher specimen present in the herbarium.

2.2. Experimental Animals

All the experimental animals used were obtained from the animal house of the Zoological Garden, University of Nigeria Nsukka.

2.3. Preparation of *Monodora Tenuifolia* Seed Flour

The fresh seeds of *Monodora tenuifolia* (**Fig. 1**) were shade dried for 48 h, then reduced in size to a coarse texture using a manual blender (Corona, Landers Colombia) and packed in airtight containers.

2.4. Proximate Analysis *Monodora Tenuifolia* Seed

Crude fat was extracted using the Soxhlet method with petroleum ether (40-60°C) for 6 h. Crude protein content was determined by microkjedahl method. These as well as carbohydrate content, crude fibre, moisture content and ash content were estimated using the method described by the Association of Official Analytical Chemists (AOAC, 2012).

Fig. 1. *Monodora tenuifolia* seeds

2.5. Mineral analysis of *Monodora tenuifolia* Seed

The mineral contents namely Sodium, Calcium, Iron, Zinc, Potassium, Magnesium, Copper and Manganese were estimated by the use of atomic absorption spectrophotometer, Phosphorus was determined according to the method of AOAC (2012).

2.6. Vitamin Analysis of *Monodora tenuifolia* Seed

Vitamin A, vitamin C, vitamin E, Riboflavin, Thiamine and Niacin were estimated using the methods described by AOAC (2012).

2.7. Determination of Amino Acid Profile

The amino acid profile of *Monodora tenuifolia* seed protein hydrosylate was determined using the method described by Ekeanyanwu (2013).

2.8. Determination of Some Antinutrients

The concentrations of some antinutrients in *Monodora tenuifolia* seed were analysed using standard laboratory procedures. Oxalate, phytate, trypsin inhibitors and total phenols were determined by the method described by Ekeanyanwu *et al*. (2010).

2.9. Extraction of Oil

A quantity, 200 g of ground seed of *Monodora tenuifolia* were extracted with petroleum ether (Merck) at 60°C using a soxhlet apparatus for 6 h. The extract was dried *in vacuo* on a rotary evaporator at 35°C yielding lipid samples as the residue.

2.11. Extraction of Flavonoid Rich Fraction

The aqueous-alcoholic extract and fractions of ground *Monodora tenuifolia* seed was obtained by

solvent-solvent extraction technique according to the method described by Harborne (2008) with slight modifications. A quantity, 5.5 kg of ground seeds of *Monodora tenuifolia* was macerated twice with 10 litres of 70% ethanol for 72 h at room temperature. The combined macerate was passed through whatman No.4 filter paper and mixed thoroughly with 1.8 L of chloroform to partition the aqueous-alcoholic extract. Two distinct layers were obtained, the upper aqueous layer and the lower chloroform layer. The two layers were drawn out separately and the chloroform fraction was dried *in vacuo*, weighed and called the Chloroform Fraction (CLF). A quantity, 2.75 L of the main (aqueous portion) extract was extracted three times with an equal volume of ethyl acetate for 48 h at room temperature. The ethyl acetate soluble extracts were pulled together and dried *in vacuo*, weighed and called the Ethyl Acetate Fraction (EAF). The remaining aqueous extracts were stored away. 11.6 g of the dried ethyl acetate fraction was then suspended in a relatively small volume (200 mL) of absolute methanol and allowed to settle. The dissolved part was separated from the rest by filtration through whatman No.4 filter paper and then concentrated *in vacuo* weighed and called Methanol Fraction (MEF). The total flavonoid content of the different fraction was determined quantitatively by spectrophotometer method.

2.12. Total Flavonoid Concentration of Fractions

The total flavonoid content of the fractional extracts and standard was determined according to the method of.

2.13. Animal Studies

2.13.1. Toxicity Study (LD_{50}) of the Flavonoid Rich Fraction

Acute toxicity study of the flavonoid rich fraction was carried out according to the method of Lorke, D. as described by (Oduola *et al*., 2010) using 39 albino mice of both sexes of average weight between 13.2 g-19.2 g, that were dosed orally with different gradual doses (10-5000 mg kg^{-1} body weight). In the first phase, mice were divided into three groups of nine mice each and were treated with the extract at doses of 10, 100 and 1000 mg kg^{-1} body weight orally by means of a cannula. They were observed for 24 h for signs of toxicity, mortality and general behaviours. In the second phase, twelve mice were divided into four groups of three mice each and were also administered with the flavonoid rich fraction at doses of 1000, 1600, 2900 and 5000 mg kg^{-1} body weight orally. They were observed for 24 h for signs of toxicity, mortality and general behaviours. The median Lethal Dose (LD_{50}) was calculated as the geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred):

$$LD_{50} = \sqrt{\text{minimum toxic dose} \times \text{maximum tolerated dose}}$$

2.14. Experimental Animals

Adult male wistar rats of age 8 weeks were used for the sub-acute toxicity profiling. The animals were obtained from the animal house of the Zoological garden, University of Nigeria, Nsukka. They were fed *ad libitum* with standard feed (Finishers mash, Premier feeds mills company limited) and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12 h light/dark cycle. The animals were acclimatised for a week before the commencement of the study. A standard protocol was drawn up in accordance with current guidelines for the care for laboratory animals and ethical guidelines for investigations of experiments in conscious animals.

2.15. Feeding Experiment

Twenty four albino rats of average weight (between 95 g to 140 g) were selected by stratified randomisation and then divided into four groups of six each. Groups I, II and III were given 100, 200 and 400 mg kg^{-1} body weight respectively of the flavonoid rich fraction orally for 14 days and 28 days respectively. 1% DMSO served as the vehicle and was used to prepare the doses. Group IV served as the control group and received the diluted vehicle only. The first day of dosing was taken as day 0 and blood was collected on day 14 and 28 respectively and used for blood glucose and lipid profile study.

2.16. Mortality and Clinical Signs (General Behaviour)

During the four-week dosing period, all the animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing and up to two to four hours after dosing.

2.17. Weekly Body Weight Measurement

The body weight of each rat was expressed using a sensitive balance during the acclimatisation period, once before commencement of dosing, once weekly during the period and once on the day of sacrifice.

2.18. Effect of the Flavonoid Rich Fraction on Blood Glucose and Lipid Profile of Rats

The values of Blood glucose, Serum cholesterol, Serum High Density Lipoprotein (HDL), Serum Low Density Lipoprotein (LDL) and Serum Very Low Density Lipoprotein (VLDL) were determined following standard laboratory procedures. All haematological parameters were determined at room temperature (27±0.5°C).

2. 19. Statistical Analysis

All analysis was conducted using Statistical Tool for Social Sciences (SPSS) for windows 7 software programme (version 17.0).Values of treated groups were compared statistically with control by Independent Sample t-test, one-way ANOVA and Spearman correlation. Inferences were made from findings at 95 and 99% confidence level. Data obtained were presented as mean ± standard deviation and analysed by simple percentages.

3. RESULTS

3.1. Proximate, Vitamins, Minerals And Antinutrients Composition Of *Monodora tenuifolia* Seed

Table 1 shows the results of the proximate analysis, vitamins and minerals of *Monodora tenuifolia* seed. *Monodora tenuifolia* seed contains crude fat, ash, crude fibre, moisture, crude protein and carbohydrate. The least abundant minerals were Mn, Cu, Zn and Fe while Na was found to be the most abundant. The vitamin C content was very high. The contents of vitamin A, E as well as Thiamine, Niacin and Riboflavin were relatively low. *Monodora tenuifolia* seed contains total phenols, oxalates, phytates and trypsin inhibitor.

3.2. Amino Acid Composition (G/Kg Crude Protein) Of *Monodora tenuifolia* Seed Protein Hydrosylate

Table 4 shows the amino acid value of *Monodora tenuifolia* seed (g/kg crude protein). The major abundant amino acids were glutamic acid, aspartic acid and leucine.

Table 5 shows the classification of the different amino acid composition (gkg^{-1} crude protein) of *Monodora tenuifolia* seed. The Total Amino Acid (TAA) was 717.8 gkg^{-1}. The percentage Total Non-Essential Amino Acid (TNEAA) (59.26 %) was higher than the percentage Total Essential Amino Acid (TEAA) (37.66%).

Essential Amino Acid (EAA), PAAESP-Provisional Amino Acid (Egg) Scoring pattern, EAAC-Essential Amino Acid Composition, AMSS-Amino Acid Scores, ND-not determined. **Table 6** shows the amino acid score of *Monodora tenuifolia* seed based on the WHO standard. *Monodora tenuifolia* seed is adequate only in the Leucine and Phenylalanine and Tyrosine contents.

3.3. Fatty Acid Composition (g/100 g Crude Protein) OF *Monodora tenuifolia* SEED

Table 6 shows the fatty acids present in the *M. tenuifolia* seed oil which are Palmitic acid (122.61 g kg^{-1}), Stearic acid (102.3 g kg^{-1}), Oleic acid (346.1 g kg^{-1}) and Linoleic acid (401.7 g kg^{-1}).

3.4. Lipid Profile And Blood Sugar Level In Rats

Values are represented as Mean±SD of triplicates. Values on the same row followed by superscript letter differ significantly ($^{a}p<0.05$, $^{b}p<0.01$) from the control. High Density Lipoprotein (HDL), Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL). **Table 6** shows the effect of the flavonoid rich fraction of *Monodora tenuifolia* seed extract on the other biochemical parameters. After 14 days of administration, triacylglycerol and very low density lipoprotein (VLDL) were significantly ($p<0.05$) increased in group III and also significantly increased ($p<0.01$) in group IV when compared with the control group. High Density Lipoprotein (HDL) was significantly ($P<0.05$) decreased in group II, III and IV when compared with the control group. Total cholesterol concentration increased slightly in group II and group III but not significantly when compared with the control. There was no significant effect ($p>0.05$) on the fasting blood sugar and Low Density Lipoprotein (LDL) level in all the treated groups when compared with the control group. After 28 days of treatment, triacylglycerol concentration was increased in all the groups but not significantly ($p>0.05$) different from the control group. High Density Lipoprotein (HDL) was increased in all the treated groups but not statistically significantly ($p>0.05$) different from the control. However, fasting blood sugar, total cholesterol and Low Density Lipoprotein (LDL) were not significantly ($p>0.05$) affected in all the treated groups compared to the control group.

Table 1. Proximate, vitamins, minerals and antinutrients composition of *Monodora tenuifolia* seed

Parameters	Composition
Crude Fibre (gkg^{-1})	262.3±1.2
Crude Protein (gkg^{-1})	82.6±1.0
Crude Fat (gkg^{-1})	349.9±1.9
Ash (gkg^{-1})	49.9±0.6
Moisture (gkg^{-1})	190.0±0.00
Carbohydrate (gkg^{-1})	65.5±4.7
Vitamin A (iu /100 g)	10.05±0.17
Vitamin C (gkg^{-1})	56.40±0.14
Vitamin E (iu /100 g)	11.71±0.87
Thiamine (gkg^{-1})	0.11±0.01
Niacin (gkg^{-1})	0.46±0.32
Calcium (Ca) (gkg^{-1})	864.0±29.38
Sodium (Na) (gkg^{-1})	2752.0±140.35
Iron (Fe) (gkg^{-1})	3.34±0.06
Zinc (Zn) (gkg^{-1})	5.26±0.08
Potassium (K) (gkg^{-1})	326.4±13.06
Magnesium (Mg) (gkg^{-1})	342.9±13.71
Copper (Cu) (gkg^{-1})	2.56±0.08
Manganese (Mn) (gkg^{-1})	1.46±0.03
Total phenols (gkg^{-1})	0.80±0.00
Oxalates (gkg^{-1})	4.090±1.17
Phytates (gkg^{-1})	0.012±0.42
Trypsin inhibitors (iu/g)	0.230±0.42

Table 2. Amino acid composition (gkg^{-1} crude protein) of *Monodora tenuifolia* protein seed hydrosylate

Amino acid	*Monodora tenuifolia*
Lysine (Lys)[a]	34.8±1.20
Histidine (His)[a]	22.1±0.40
Arginine (Arg)[a]	59.1±0.42
Aspartic acid (Asp)	100.9±7.70
Threonine (Thr)[a]	31.0±2.10
Serine (Ser)	27.8±3.20
Glutamic acid (Glu)	118.9±4.30
Proline (Pro)	31.5±1.40
Glycine (Gly)	32.2±9.80
Alanine (Ala)	39.7±1.80
Cysteine (Cys)	10.3±1.40
Valine (Val)	33.9±3.20
Methionine (Met)[a]	9.2±1.60
Isoleucine (Ile)[a]	30.6±1.90
Leucine (Leu)[a]	60.9±1.50
Tyrosine (Tyr)	30.2±2.20
Phenylalanine (Phe)[a]	44.7±2.40
(P-PER)	19.8

[a]; Essential amino acid, P-PER-calculated predicted protein efficiency ratio

Table 3. Classification of amino acid composition (gkg^{-1} crude protein) of *Monodora tenuifolia* seed

Amino acid	*Monodora tenuifolia* seed
Total Amino Acid (TAA)	717.80
Total Non-Essential Amino Acid (TNEAA)	425.40
%TNEAA	59.26%
Total Essential Amino Acid (TEAA) (with His)	292.40
TEAA (without His)	270.30
%TEAA (with His)	40.74%
%TEAA (without His)	37.66%
Essential Aliphatic Amino Acid (EAAA)	125.40
Essential Aromatic Amino Acid (EArAA)	44.70
Total Neutral Amino Acid (TNAA)	58.80
%TNAA	8.19%
Total Acidic Amino Acid (TAAA)	219.80
%TAAA	30.62%
Total Basic Amino Acid (TBAA)	93.90
%TBAA	13.08%
Total Sulphur Amino Acid (TSAA)	19.50
% of Cysteine in TSAA	52.82%

Table 4. Amino acid score of *Monodora tenuifolia* seed

EAA	PAAESP[a]	EAAC	AMSS
Ile	4.0	3.06	0.77
Leu	7.0	6.09	0.87
Lys	5.5	3.48	0.63
Met + Cys (TSAA)	3.5	1.95	0.56
Phe + Tyr	6.0	7.49	1.25
Thr	4.0	3.10	0.78
Try	1.0	ND	ND
Val	5.0	3.39	0.68
Total	36.0	28.56	5.54

Table 5. Fatty acid profile of *Monodora tenuifolia* seed oil

Fatty acid	Composition (g/kg)
$C_{8:0}$	0.21
$C_{10:0}$	0.22
$C_{11:0}$	0.14
$C_{12:0}$	0.34
$C_{14:0}$	0.33
$C_{16:0}$	122.60
$C_{16:1}$	1.33
$C_{18:0}$	102.30
$C_{18:1\ n-9}$	346.10
$C_{18:2\ n-6}$	401.70
$C_{18:3\ n-3}$	1.30
$C_{18:3\ n-6}$	12.90
$C_{20:1}$	7.31
$C_{20:3\ n-3}$	0.60
$C_{20:3\ n-3}$	1.10
Total saturated	226.15
Total unsaturated	772.34
Unknown	7.90
Unsaturated/saturated	3.42

Table 6. Levels of serum lipids and blood sugar in rats on flavonoid rich fraction of *Monodora tenuifolia* seed extract

Parameter	Group I Control	Group II 100 mg kg^{-1}	Group III 200 mg kg^{-1}	Group IV 400 mg kg^{-1}
Day 14, n = 3				
Fasting Blood Sugar (mg/dL)	88.33±15.50	85.33±6.65	95.00±13.89	95.33±12.08
Total Cholesterol (mg/dL)	98.65±8.42	100.38±5.78	107.12±3.84	86.51±15.26
HDL (mg/dL)	35.85±1.21	29.84±2.62[a]	29.07±1.53[a]	29.26±2.21[a]
Triacylglycerol (mg/dL)	37.72±3.62	45.61±12.89	78.51±17.96[a]	84.54±18.14[b]
VLDL (mg/dL)	7.54±0.73	9.12±2.57	15.70±3.59[a]	16.90±3.62[b]
LDL (mg/dL)	55.25±7.95	61.41±6.20	62.35±7.49	48.83±9.07
Day 28, n = 3				
Fasting Blood Sugar (mg/dL)	93.66±7.63	88.66±9.07	89.66±10.70	95.00±11.53
Total Cholesterol (mg/dL)	93.67±6.52	99.80±5.46	95.11±13.41	90.03±6.85
HDL (mg/dL)	29.56±2.63	29.50±5.58	27.87±0.40	30.84±2.71
Triacylglycerol (mg/dL)	62.15±6.70	66.77±16.60	74.71±4.68	59.06±11.38
VLDL (mg/dL)	12.43±1.33	13.35±3.32	14.94±0.93	11.81±2.27
LDL (mg/dL)	57.70±3.06	57.13±4.49	52.30±12.07	47.37±6.22

4. DISCUSSION

The percentage crude fibre content of *Monodora tenuifolia* seed was higher than values reported for *Xylopiaa ethiopica*, *Tetrapleura tetraptera*, *Piper guineense* (Effiong *et al*., 2009) and *Monodora myristica* (Ekeanyanwu *et al*., 2010). This quality of *Monodora tenuifolia* is highly desirable because adequate intake of dietary fibre can lower serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ekeanyanwu *et al*., 2010).

The percentage crude protein content was comparable to the reported values of some local Nigerian spices in literature (Ekeanyanwu *et al*., 2010; Effiong *et al*., 2009). This indicates that *Monodora tenuifolia* seed is a good source of dietary protein supplement to meet the recommended daily requirements for human (Ekeanyanwu *et al*., 2010). The importance of protein to animals and human health cannot be over emphasized therefore, *Monodora tenuifolia* seeds may be used both as feed and food protein supplements.

The seeds contained crude fat which is higher than the values reported for some local Nigerian spices (Ekeanyanwu *et al*., 2010; Effiong *et al*., 2009). Crude fats are the principal sources of energy; it also promotes fat soluble vitamins absorption (Ekeanyanwu *et al*., 2010) but should be consumed with caution so as to avoid obesity and other related disease.

The ash content of the seeds shows that it is rich in mineral element and higher than that reported for some local Nigerian spices (Ekeanyanwu *et al*., 2010; Effiong *et al*., 2009). It has been recommended that ash contents of nuts, seeds and tubers should fall within the range of 1.5-2.5% in order to be suitable for animal feeds (Ekeanyanwu *et al*., 2010). The ash content of *Monodora tenuifolia* seed does not fall within the range and hence it may not be suitable for animal feeds.

The moisture content was higher than values reported for *Piper guineense* and *Monodora myristica* (Ekeanyanwu *et al*., 2010; Effiong *et al*., 2009) but lower than those reported for *Xylopia aethiopica* and *Tetrapleura tetraptera* (Effiong *et al*., 2009). The low moisture content of the seeds would hinder the growth of micro organisms and shelve life could be higher (Ekeanyanwu *et al*., 2010).

The carbohydrate content of the seeds is considerably low compared to some local Nigerian spices.

The mineral composition of *Monodora tenuifolia* seed flour is contained in **Table 1**. The least abundant minerals were Mn, Cu, Zn and Fe while Na was found to be the most abundant. Calcium was found to be the next highest mineral component. Calcium in conjunction with phosphorus, magnesium, manganese, vitamin A, C and D, chlorine and protein are involved in blood formation (Ekeanyanwu *et al*., 2010). Calcium is also important in blood clothing, muscle contraction and in certain enzymes in metabolic processes. The calcium concentration in *Monodora tenuifolia* is higher than values reported for some local Nigerian spices (Ekeanyanwu *et al*., 2010; Effiong *et al*., 2009). Considering the importance of calcium for the growth and maintenance of bones, teeth and muscles, this value could be said to be adequate since it can contribute 72% to the Recommended Daily Allowance (RDA) values of calcium for an adult man with 300 kcal/day whose recommended energy intake is 1200 mg (Ekeanyanwu *et al*., 2010). This implies that calcium can moderately contribute to the amount of dietary calcium. The phosphorus content was low.

Calcium and phosphorus are the minerals present in the largest quantity in the structure of the body and bones. A food source is considered good if the Ca/P is above 1 and poor if the ratio is less than 0.5 (Aremu *et al*., 2011). *Monodora tenuifolia* seeds with 90.76 Ca/P are therefore a good source of minerals needed in bone formation.

The potassium content of *Monodora tenuifolia* is higher than values found in local spices like *Xylopia ethiopica*, *Tetrapleura teraptera* and *Piper guineense* but lower than in *Monodora tenuifolia* (Ekeanyanwu *et al*., 2010; Effiong *et al*., 2009). However, this value is small and can contribute little to the body requirements of dietary potassium. The seeds of *Monodora tenuifolia* can only contribute about 16.3% of the Recommended Daily Allowance (RDA) of potassium which is 2000 mg for adults (USDA/HHS, 2010).

The sodium content of *Monodora tenuifolia* seed was higher than that reported for most local Nigerian spices (Ekeanyanwu *et al*., 2010; Effiong *et al*., 2009). *Monodora tenuifolia* seeds can contribute about 550 % to RDA since the RDA value of sodium for adult is 500 mg (USDA/HHS, 2010). The importance of Na/K ratio in the body in controlling high blood pressure cannot be over emphasized. *Monodora tenuifolia* seeds as spices in diets could be useful in lowering blood pressure.

The magnesium content of *Monodora tenuifolia* seed is higher than the values reported for some local Nigerian spices in literature (Ekeanyanwu *et al*., 2010; Effiong *et al*., 2009). *Monodora tenuifolia* seeds can contribute about 97.97% to RDA since the RDA for magnesium in adult is 350 mg (USDA/HHS, 2010). This means that the seeds can serve as a good source of magnesium.

The copper content of *Monodora tenuifolia* seed is far much higher than values in *Monodora myristica* seeds (Ekeanyanwu *et al*., 2010) but lower than the values in *Xylopia aethiopica*, *Tetrapleura tetraptera* and *Piper guineense* (Effiong *et al*., 2009). *Monodora tenuifolia* seed could serve as a good source of copper in both animal and human diets since the RDA value of copper is 1.5-3 mg for a male adult and 170.66-341.33% are contributed to the RDA by *Monodora tenuifolia* (USDA/HHS, 2010).

The Iron content of *Monodora tenuifolia* seed is low compared with values reported for some local Nigerian spices in literature (Ekeanyanwu *et al*., 2010; Effiong *et al*., 2009). The importance of iron as a trace element in the body can be seen in its role in haemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrates, proteins and fats (Ekeanyanwu *et al*., 2010). This value for iron shows that *Monodora tenuifolia* seeds can contribute in boosting the blood level in anaemic conditions (USDA/HHS, 2010).

The zinc content of *Monodora tenuifolia* seed is greater than the values reported for *Piper guineense* and *Monodora myristica* (Effiong *et al*., 2009; Ekeanyanwu *et al*., 2010) but lower than values reported for *Xylopia aethiopica* and *Tetrapleura tetraptera* (Effiong *et al*., 2009). *Monodora tenuifolia* seeds can contribute about 35.1-43.8% of the RDA value of zinc which for male adult is 12-15 mg (USDA/HHS, 2010). The seeds of *Monodora tenuifolia* are a fairly good source of zinc.

The manganese content in *Monodora tenuifolia* is higher than the values reported for *Piper guineense* (Effiong *et al*., 2009) and *Monodora myristica* (Ekeanyanwu *et al*., 2010) but lower than values reported for spices like *Xylopia aethiopica* and *Tetrapleura tetraptera* (Effiong *et al*., 2009). The RDA for manganese is 2-5 mg 100^{-1} g for a male adult and *Monodora tenuifolia* seeds can contribute about 29.2-73% of manganese to the RDA and could be said to be a fairly good source of Manganese (USDA/HHS, 2010).

The contents of vitamin A, E as well as Thiamine, Niacin and Riboflavin were relatively low. The recommended daily allowance for vitamin A is 3000 iu/l for children and 5000 iu/l for adults (USDA/HHS, 2010). The vitamin A level of *Monodora tenuifolia* seed can supply the recommended daily allowance for the vitamin. Vitamin A content of *Monodora tenuifolia* is comparable with the vitamin A concentration of other spices reported in literature (Uhegbu *et al*., 2011). The vitamin C content of *Monodora tenuifolia* seed is comparable with the values reported for other local spices in literature (Uhegbu *et al*., 2011). The recommended daily allowance for vitamin C is 40-45 mg 100^{-1} g for children and 45-60 mg 100^{-1} g for adults (USDA/HHS, 2010). The Vitamin C content of *Monodora tenuifolia* seed can sufficiently supply the recommended daily allowance for the vitamin.

The vitamin E content of *Monodora tenuifolia* seed is comparable with the vitamin E content of other spices such as *Monodora myristica* and *Tetrapleura tetraptera* (Uhegbu *et al*., 2011), but higher than that of other spices such as *Gongronema latofollium*, *Xylopia aethiopica* and *Piper guineense* (Uhegbu *et al*., 2011). The recommended daily allowance for vitamin E is 10 iu/l for children and 15 iu/l for adults (USDA/HHS, 2010). The vitamin E contents of *Monodora tenuifolia*

will sufficiently supply the recommended daily allowance of the vitamin in our diet.

Table 2 showed the amino acids values of *Monodora tenuifolia* seed protein hydrosylate of the seed. The major abundant amino acids were glutamic acid, aspartic acid and leucine. This value compare favourably with values obtained for selected spices (pepper, garlic, ginger, onion, curry leaf, tomatoes and African nutmeg) ranging from 39.21-78.08 (Ekeanyanwu, 2013).

Leucine was the most concentrated essential amino acid in *Monodora tenuifolia* (**Table 2**). The leucine concentration of *Monodora tenuifolia* is comparable with values obtained for spices like pepper, ginger, onion, tomatoes and African nutmeg (Ekeanyanwu, 2013), but slightly lower for values obtained for other spices like garlic and curry leaf (Ekeanyanwu, 2013). This concentration of leucine in *Monodora tenuifolia* is in agreement with the observations made earlier by some researchers (Ekeanyanwu, 2013) that leucine is the most concentrated essential amino acid in Nigerian plant products. Glutamic acid was the most concentrated amino acid in *Monodora tenuifolia* seed.

The Predicted Protein Efficiency Ratio (P-PER) is one of the quality parameters used for protein evaluation (FAO/WHO, 2002). The P-PER of *Monodora tenuifolia* was 1.98. This value compared favourably with P-PER value of some spices (Ekeanyanwu, 2013).

The total essential amino acid (with His) of *Monodora tenuifolia* was found to be 29.24 (**Table 3**). This is comparable with values obtained for spices (Ekeanyanwu, 2013) suggesting that *Monodora tenuifolia* can effectively serve as a food supplement. Essential Aliphatic Amino Acid (EAAA), Ile, Leu and Val content which constitutes the hydrophobic regions of proteins was found to be 12.54. This is slightly lower than values for some spices (Ekeanyanwu, 2013). This means that better emulsification property may not be expected in *Monodora tenuifolia* seed flour compared with other spices such as pepper and curry leaf. **Table 5** also depicts that the percent of Total Acidic Amino Acid (TAAA) of *Monodora tenuifolia* was greater than the percent of Total Basic Amino Acid (TBAA) indicating that the protein is probably acidic in nature. The TSAA is lower than 58 g kg^{-1} crude protein recommended for infants (FAO/WHO/UNU, 2001).

The content of some essential amino acids was lower than FAO/WHO (2002) recommendation (**Table 4**). However, *Monodora tenuifolia* was adequate only in Leu and Phe + Tyr. Thus based on the finding, *Monodora tenuifolia* may be used as a food supplement for any food material that is not adequate in essential amino acid. It has been reported that the essential amino acids most often acting in a limiting capacity are Met (and Cys), Lys and Try (Ekeanyanwu, 2013). The first limiting amino acid in this study was Met+Cys. Tryptophan (Try) could not be determined.

This study showed that *Monodora tenuifolia* contained nutritionally useful quantities of most of the essential amino acids and can serve as food supplement for food materials that are not adequate in essential amino acid based on FAO/WHO provisional pattern.

The result of Antinutritive factors in the analysed seed is contained in **Table 1**. The phytate content of *Monodora tenuifolia* seed is low compared to other related spice like *Monodora myristica* (Ekeanyanwu *et al*., 2010). High phytate content could decrease the bioavailability of minerals especially Ca, Mg, Fe and Zn (Ekeanyanwu *et al*., 2010).

The analysed *Monodora tenuifolia* seed oxalate content is high compared to the related spice like *Monodora myristica* (Ekeanyanwu *et al*., 2010). Presence of oxalates in food causes irritation in the mouth and interfere with absorption of divalent minerals particularly calcium by forming insoluble salts with them (Ekeanyanwu *et al*., 2010).

Phenols have been extremely reported as disease preventive (Cartea *et al*., 2011). Phenols content of *Monodora tenuifolia* seed could further indicate its ability to act as antiiflammatory, anticlotting, antioxidant and immune enhancer.

Trypsin inhibitor content of *Monodora tenuifolia* is very low. High levels of the trypsin inhibitor have been reported to result in growth retardation by not only impairing protein digestion but also due to the endogenous loss of essential amino acids (Awogbenja and Ugwuona, 2012).

Monodora tenuifolia seed oil contains high level of unsaturated fatty acids which is about 772.34 g kg^{-1}. *Monodora tenuifolia* seed oil compare favourably with that of some vegetable oils (Dauqan *et al*., 2011) indicating that the plant is rich in fatty acids such as Oleic acid (C18:1), Linoleic acid (C18:2) and Eicosapentanoic acid (C20:5). *Monodora tenuifolia* seed oil has a relatively high level of Polyunsaturated Fatty Acids (PUFA), Trienes (γ-Linoleic acid, GLA, (18:3n-6) and (α-Linoleic acid, ALA, C18:3n-6) as well as EPA (C20:5) were also found in relatively lower amounts. A rapid growing literature illustrates the benefits of PUFA in alleviating cardiovascular conditions, heart disease, atherosclerosis, auto immune disorders, diabetes and other diseases (NHF, 2008).

Chloroform was the highest extracting solvent amongst the other solvents used in preparation of the flavonoid rich fraction because it generally extracted the highest quantity of plant material from the aqueous-alcoholic extract compared to ethyl acetate. It shows that the seeds of *Monodora tenuifolia* contain more non polar material than polar material since chloroform extracted the highest quantity of plant material. The ethyl acetate fraction had the highest content of total flavonoid and was used as the flavonoid rich fraction in subsequent animal studies.

In the acute toxicity study, flavonoid rich fraction of *Monodora tenuifolia* seed did not show any mortality up to the dose of 5 g kg^{-1} during the observational period of 48 h. However, minor changes in behaviour, breathing and activity were observed in mice administered 1600, 2900 and 5000 mg kg^{-1} of the extract few minutes after administration of th e extract. These results showed that in single dose, there was no adverse effect of flavonoid rich fraction of *Monodora tenuifolia* seed extract, indicating that the medium Lethal Dose (LD_{50}) is higher than 5000 mg kg^{-1} for both male and female mice. Accordingly, about one-twelfth of the maximum tolerated dose, that is 400 mg kg^{-1} was considered as the high dose of the flavonoid rich fraction of *Monodora tenuifolia* seed extract and used for the subsequent animal studies.

The results of the mortality and gross symptoms of toxicity seen in rats administered with the flavonoid rich fraction of *Monodora tenuifolia* seed extract over 28days showed there was no noticeable deviation in the behaviour of the rats treated with 100, 200 and 400 mg kg^{-1} compared to that of the control (Group I, No dose) group and essentially all the treated rats remained healthy during the 28 days period of oral administration of the flavonoid rich fraction of *Monodora tenuifolia* seed aqueous-alcoholic extract. Moreover, no deaths occurred with any of the doses up to 400 mg kg^{-1} given over 28 days confirming that the LD_{50} for sub acute dosing with ethyl acetate extract of *Monodora tenuifolia* seed was higher than 400 mg kg^{-1}.

The mean body weight change in rats after every seven days following administration of 100, 200 and 400 mg kg^{-1} b. w. flavonoid rich fraction of *Monodora tenuifolia* seed aqueous-alcoholic extract increased. A general increase in physical activities, food and water intake were observed for all the animals during the feeding experiment. There was slight increase in weight which peaked subsequently suggesting that the extract does not exert any deteriorative effect on the weight and growth of the animals. The increased weight could be due to increased feed and water intake observed all through the experimental period. The increase in weight of the animals suggests that they increasingly accumulated calories from the normal rat diet. Although the animals used in this study were fed with normal rat diet, the *Monodora tenuifolia* seed extract might have allowed proper absorption and utilisation of the nutrients. Low level of active/toxic principles may have stimulated appetite and increased feed utilization resulting in increased weight gain. The seed of *Monodora tenuifolia* is used as a spice (Ezenwali *et al*., 2010) and there have not been any reported cases of toxicity in humans.

The liver, being a key organ in the metabolism and detoxification of xenobiotics, is vulnerable to damage induced by a huge variety of chemicals (Kozat and Denizhan, 2010). There was significant ($p<0.05$) increase in liver weight of the group treated with 400 mg kg^{-1} body weight of the flavonoid rich fraction after the 14 day study which could be attributed to high rate of metabolism of the liver resulting from the brief exposure to substance (containing several constituents).

The result of the fasting blood sugar and lipid profile study after 14 days of administration of the flavonoid rich fraction showed that the fraction administered to rats caused a significant increase in the serum levels of Triacylglycerol (TAG) and Very Low Density Lipoprotein (VLDL) in groups administered 100 mg kg^{-1} (group II), 200 mg kg^{-1} (group III) and 400 mg kg^{-1} (group IV) of the flavonoid rich fraction while serum High Density Lipoprotein (HDL) reduced significantly in groups administered 100 mg kg^{-1} (group II), 200 mg kg^{-1} (group III) and 400 mg kg^{-1} (group IV) of the flavonoid rich fraction of *Monodora tenuifolia* seed extract. There was no effect on fasting blood sugar level and lipid profile parameters like Low Density Lipoprotein (LDL) and cholesterol in the groups administered 100 mg kg^{-1} (group II), 200 mg kg^{-1} (group III) and 400 mg kg^{-1} (group IV) of the flavonoid rich fraction of *Monodora tenuifolia*. Although the flavonoid rich fraction caused a significant decrease in the serum level of HDL, which could impact a corrective effect on the incidence of coronary heart disease (Feray and Ben, 2010), the observed significant increase in the triacylglycerol and very low density lipoprotein seems to suggest atherogenic potential for the flavonoid fraction of *Monodora tenuifolia* seed. The mechanism might be

connected with the effects of the phytochemical constituents of the extract on the lipid biosynthetic machinery with the body. The results obtained in this study clearly show that the spice examined exerted an initial effect on serum triacylglycerol, very low density lipoprotein and high density lipoprotein which eventually normalised after 28 days of oral administration of the flavonoid rich fraction implies that the impairment of lipid metabolism was impermanent.

5. CONCLUSION

From the results obtained in this study, it can be concluded that *Monodora tenuifolia* seed is rich in important nutrients especially crude fat and crude fibre which are higher than values reported for most spices in literature. *Monodora tenuifolia* seed is rich in important minerals with sodium and calcium being more abundant than the other minerals analysed. *Monodora tenuifolia* seed is rich in important vitamins like Vitamins A, C and E and also contains fewer amounts of antinutrients compared with other spices in literature. The amino acid content of *Monodora tenuifolia* protein hydrosylate in the seed is adequate with acidic amino acids like glutamic acid and aspartic acid being most abundant. *Monodora tenuifolia* seed may be suitable as edible oil. *Monodora tenuifolia* seed appears to be practically safe (LD_{50} above 5000 mg kg^{-1}) when administered acutely to mice through the oral route. When administered orally to rats for the 14 and 28 day study, some biochemical parameters like serum triacylglycerol and very low density lipoprotein were significantly increased after 14 days of administration. However, these parameters normalised after 28 days of administration of the flavonoid rich fraction.

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