



GLOBAL JOURNAL OF SCIENCE FRONTIER RESEARCH
CHEMISTRY
Volume 13 Issue 1 Version 1.0 Year 2013
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

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By Ibrionke A. Ajayi, Emmanuel Ifedi & Vivian N. Aghanu

University of Ibadan

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Keywords : *amino acid; garcinia mangostana; mineral element; proximate; phytochemical; toxicological effect.*

GJSFR-B Classification: FOR Code: 070204, 110101



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Amino Acid Analysis and Preliminary Toxicological Evaluation of Garcinia Mangostana Seed Cake in Albino Rats

Ibironke A. Ajayi^α, Emmanuel Ifedi^σ & Vivian N. Aghanu^ρ

Abstract - The amino acid analysis and preliminary toxicological effect of *Garcinia mangostana* seed cake on albino rats have been investigated in order to determine its suitability as an additive in feed supplement. The proximate analysis of the seed cake showed that it had a high carbohydrate value of $71.02 \pm 0.79\%$ but low protein, moisture, fat and ash contents of $8.09 \pm 0.21\%$, $10.42 \pm 1.12\%$, $2.13 \pm 0.13\%$ and $1.8 \pm 0.02\%$ respectively. The seed cake also contains manganese, calcium, potassium, sodium, magnesium, iron, copper and zinc. The result of the phytochemical analysis revealed that *Garcinia mangostana* seed cake contains tannins, flavonoids and alkaloids. Among the seventeen amino acid determined in the defatted seed, glutamate gave the highest concentration of $10.94 \text{ g}/100\text{g}$. During the rat experiment which lasted for six weeks, both the control and test rats appeared to suffer no toxicological effect and weekly monitoring showed good physical appearance. There was no significant difference between the control and test groups for the haematology and histopathology examination of sections of the heart, liver, kidney, spleen, lung, intestine and brain result.

Keywords : amino acid; *Garcinia mangostana*; Mineral element; proximate; phytochemical; toxicological effect.

I. INTRODUCTION

Garcinia *mangostana*, a Clusiaceas, commonly known as mangosteen and one of the most universal recognized tropical fruit, has universal appeal because of its quality in colour, shape and flavour. It is a tropical evergreen tree originated from South East Asia, probably Malaysia; it can also be seen in India, Myammer, Philippine, Srilanka, Thailand and even in Nigeria. The tree can reach 6–25 m and has leathery, glabrous leaves and is slow to grow (Morton, 1987). The fruits are dark purple or reddish with white soft and juicy edible pulp with a slightly acid and sweet flavor and a pleasant aroma (Jung *et al.*, 2006). The white juicy edible pulp also has high sugar content (Kanchanapoom and Kanchanapoom, 1998; Plartin, 1980 and Nakasone and Paul, 1998). *G. mangostana* is also known as “Queen of Fruit” because it is one of the best tasting tropical fruits. The timber is dark brown, rather hard and heavy and the inner dark yellowish

petioles of *G. mangostana* tree are short and thick. The flowers are 5 cm in diameters, four parted bisexual and borne singles or in pairs at the end of the branchlets. The seeds are large, flattened and embedded in snow white or pinkish delicious pulp botanically called “aril” edible flesh which can be described as sweet, tangy, citrusy with peach flavor and texture (Abbiw, 1990). The fruit mangosteen is rated as one of the most delectable of the tropics and the pulp gives the fruits its reputation as one of the finest and most delicious of fruits. Good fruits may attain 6–7 cm in diameter and contain 5–7 seeds surrounding a white, sweet and succulent flesh (Burkill *et al.*, 1994). *G. mangostana* is an emerging category of novel functional foods sometimes called super fruit presumed to have a combination of appealing subject characteristics such as taste, fragrance, visual qualities, nutrient richness, antioxidant strength (Primchamien, 2004) and potential impact for lowering risk of human diseases (Jose *et al.*, 2008) and the pericarp is widely useful. *G. mangostana* has some medicinal properties; it possesses antitumor, antimicrobial, antifungal, anti-inflammatory and antioxidative properties (Chairrungsri *et al.*, 1996).

We are living in a world where maximum utilization of natural resources is the ultimate goal. Currently, there is little information on the *Garcinia mangostana* seed cake. The seed is neither eaten nor used for any industrial purposes in Nigeria. Apart from the chemical composition of *G. mangostana* seed and its oil and the preliminary toxicological evaluation of the oil carried out by Ajayi *et al.*, 2007, there is no literature report on the defatted seeds hence the need for this research. The aim of this work, therefore, is to determine the amino acid and mineral element composition, carry out phytochemical screening, and evaluate the toxicological effect of using defatted *G. mangostana* seeds as feed supplement in albino rat feed.

II. MATERIALS AND METHODS

a) Materials

Garcinia mangostana fruits used for this work were collected from the Botanical Garden of the University of Ibadan; Oyo State, Nigeria. The seeds were removed from the fruits, washed with water and left to air dry for two days.

Author α σ ρ : A Industrial Unit, Chemistry Department, Faculty of Science, University of Ibadan, Ibadan, Nigeria.
E-mail : frajayi@yahoo.com

University of Ibadan; Oyo State, Nigeria. The seeds were removed from the fruits, washed with water and left to air dry for two days.

b) Sample Preparation

The seeds of *G. mangostana* were decorticated manually and grounded into a paste using a previously cleaned and dried mortar and pestle. The paste was then stored in an air tight container for analysis.

c) Preparation of Defatted Garcinia Mangostana

The mangostana oil was extracted by a solvent extraction method using hexane (bp 40-60 °C) in a soxhlet extractor. *Garcinia mangostana* seed cake (GMSC) remaining after the oil extraction was air dried, pulverized and passed through a 200 mesh size to obtain the defatted powder which was used as experimental material.

d) Proximate Analysis

Percentage moisture, crude fat, ash and crude fibre contents of *G. mangostana* seed cake were determined following the methods of Association of Official Analytical Chemists (AOAC, 2006). Nitrogen content of the seed cake was estimated using the micro-kjeldahl method as described by AOAC (1984) and crude protein was calculated (N x 6.25). Carbohydrate contents were determined by difference [100 - (moisture + protein + crude fat + ash + crude fiber)].

e) Analysis of Mineral Elements

0.5 g of GMSC was digested with 20 ml mixture of concentrated HNO³ and perchloric acid (2:1 v/v) until the solution became a clean one. Thereafter, it was transferred to a 100 ml volumetric flask and diluted. It was made up to the mark with deionized water and stored in a clean polyethylene bottle. The mineral element content was determined using an atomic absorption spectrophotometer (Perkin-Elmer model 703, USA) as described by Onyeike and Acheru (2002).

f) Amino Acid Analysis

The seed cake was hydrolysed in 6M HCl at 105 °C for 22 hours in nitrogen flush. The hydrolysate was further analysed for amino acids using the sequential multi-sample amino acid analyzer as described by Spackman *et al.* (1958). The chromatogram of the sample was compared using norleucine as a standard.

g) Phytochemical Analysis

The phytochemical screening for the presence of saponins, tannins, alkaloids, flavonoid, steroid and glycoside were carried out according to the methods describe by Trease and Evans (1983) and Hassan *et al.* (2004).

h) Experimental Animals

Fourteen albino rats (aged 8 weeks; weighing between 105-115 g) were obtained from the Physiology

Department, University of Ibadan, Nigeria. The rats were divided into 2 groups of 7 rats per group named A and B for control and experimental group respectively. During the 6 weeks period of the experiment, the rats in the control group were fed the basal diet only while the ones in the experimental group were fed a diet in which 7.08% of GMSC was used to totally replace corn bran. All the rats were fed *ad libitum* and they had unrestricted access to drinking water. The feed intake and body weight gain was noted every week (Leontowicz *et al.*, 2007).

i) Feed Compoundment

A basal diet was formulated to meet the entire nutrient requirement for young albino rat of 8 weeks (*Rattus norvegicus*). GMSC was used to totally replaced corn bran in the diet formulated. The diets were prepared according to the procedure described by Souza *et al.*, (2007) with slight modification. The basic ingredients used were 2800.0 g of maize, 1274.7 g of soy bean, 231.0 g of calcium, 55.3 g of salt, 994.0 g of groundnut cake, 495.6 g of palm kernel cake, 495.6 g of wheat, 495.6 g of corn bran and 158.2 g of oyster shell for the control diet with 495.6 g of GMSC replacing corn bran in the experimental diet (Table 1). Ingredients of the diets were mixed thoroughly with the mixing machine to obtain a homogenous mixture which was pelletized and packed into two different transparent sterile plastic containers.

j) Blood Sample and Tissue Collection

At the end of the feeding period, the rats were fasted over night. Blood sample was immediately collected from the eye into bottles containing EDTA to prevent blood coagulation, and then, they were harvested. In one part of the blood, the haematological studies was carried out and to the other part, serum was separated by centrifugation. The tissues collected were kidney, heart, spleen, lungs, small intestine, brain and liver. These organs were weighed immediately after collection and preserved in formalin for pathology studies.

k) Haematological Examination

The packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts were determined using the standard techniques described by Dacie and Lewis (1991) and Jain (1986). The differential WBC counts mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated (Jain, 1986).

l) Tissue Pathology

The internal organs were exposed by dissection and the liver, spleen, kidney and lungs were observed for gross lesions. Small portions of each organ already stored in formalin were fixed and put through timed series of dehydration in graded concentrations of

xylene. They were embedded in wax, sectioned at 5 μ and transferred on to clean glass slide. The thin sections were stained with haematoxylin and eosin (H and E) dye for examination under light microscope for histological changes (Jain, 1986).

m) Statistical Analysis

Data are expressed as the means and standard errors of five separate contents, except for fatty acid. They were statistically analysed by 2-way analysis of variance (ANOVA) and means were compared by Duncan's multiple range test (Duncan, 1955) at 5 % level of significance.

III. RESULT AND DISCUSSION

a) Proximate Composition

The result of proximate analysis of GMSC is shown in Table 2 below. The ash content is $2.13 \pm 0.03\%$ and the crude fibre content was found to be $6.49 \pm 0.01\%$. The carbohydrate content is very high, $71.02 \pm 0.79\%$. This high carbohydrate content and crude fibre value suggest that the seeds could serve as source of roughage and the suitability of compounding it in animal feeds, (Abighor *et al.*, 1997).

The protein content of GMSC is very low $8.09 \pm 0.01\%$. It is lower in comparison to the values ($11-27.8 \pm 0.04\%$) reported for wheat flour and defatted wheat germ flour (Muhammad *et al.* 2007) and falls within the range of (6-12%) reported for conventional cookies (Shrestha & Noomhorm, 2002). The ash content, $2.13 \pm 0.03\%$ is lower than the value reported for *A. heterophyllus* and *T. africana* (Ajayi, 2008), and greater than the values determined for seeds such as kolanut, coconut and melon seeds (Onyeike & Acheru, 2002). Addition of GMSC resulted in an increase in the ash values of the feed compounded up to $6.79 \pm 0.02\%$, crude fibre content up to $17.73 \pm 0.31\%$ and fat content up to $9.79 \pm 0.02\%$.

b) Mineral Element Composition

Table 3 shows the mineral elements that were analysed using atomic absorption spectrophotometer on the acid digest of the defatted *Garcinia mangostana*. The result of metal composition indicates that, defatted *Garcinia mangostana* flow has high level of potassium (270 mg/L), followed by magnesium (110 mg/L), followed by iron (68.62 mg/L), and calcium (30 mg/L). Potassium is an essential mineral element which helps to regulate blood pressure, while calcium is needed for bone growth and muscle concentration. Magnesium works with calcium to maintain healthy bones and hearth. Feed compounded with GMSC will help to prevent deficiency of potassium, magnesium, iron and calcium since the seed are rich in these element (Ajayi *et al.*, 2007). *Garcinia mangostana* seed cake can be a good source of iron and slightly manganese. These minerals in the diet are generally required for metabolic

reactions, transmission of nerve impulse and rigid bone formation among others (Egwin *et al.*, 2010).

c) Phytochemical Analysis Results

Phytochemical results suggested that carbohydrate, alkaloids tannins and flavonoids can be isolated from *G. mangostana* seed cake. The result revealed that GMSC contains an array of phytochemicals which include carbohydrate, tannins, flavonoids, alkaloids and glycosides (Table 4). Saponins, steroids and terpenoids, were not observed. It is important to note that phytochemical constituent can help one to speculate on the medicinal value of the seed (Oladosu *et al.*, 2011). Tannins and alkaloids have been reported to have pronounced physiological effect particularly on the nervous system (Oladosu *et al.*, 2011). The presence of flavonoids in GMSC suggests that the seed plant is pharmacologically active, supporting the claim by traditional healers. This result obtained is comparable to the reported phytochemical components which indicate the presence of alkaloids and flavonoids in *Coffea genus* (Simkin *et al.*, 2008; Koshio *et al.*, 2006; Andrade *et al.*, 1998) and also in *Coffea brivipes* extract (Oladosu *et al.*, 2011).

d) Amino acid results

Among the 17 amino acids determined in GMSC (Table 5), glutamate gave the highest concentration of 10.938 g/100g followed by cystine 9.144 g/100g, aspartate 8.942 g/100g, leucine 6.292 g/100g and arginine 6.285 g/100g. Etonihu *et al.* (2009) stated that glutamate (14.64 g/100g), aspartate (9.85 g/100g), phenylalanine (6.85 g/100g) and arginine (6.38 g/100g) were the major constituents of amino acid in pigeon pea proteins while for defatted wheat germ, Muhammed *et al.* (2007) found glutamate, aspartate, arginine, cystine and leucine to be 5.09 g/100g, 1.63 g/100g, 4.76 g/100g, 0.00 g/100g, and 1.11 g/100g respectively.

e) Physical Appearance

Generally, the rats maintained fine and smooth hairs all through. There was no significant smell except that of their urine. Both the control and experimental group have the normal rats smell. It was important to note that no mortality was recorded throughout the period of this work.

f) Weight Changes

i. Body and Feeds Weight Changes

Figs. 1 and 2 show the body weight changes and the feed intake per week of rats in both the test and control groups. There were positive weight changes in each group within the period of this study. In group A, the weight increased from 114.29 ± 9.75 to 148 ± 5.53 while in group B, it increased from 107.14 ± 20.58 to 152.86 ± 7.55 . It is an indication of the desirability of the diet. Rats in group B displayed fairly similar body weight gain to that in group A as there was no much significant

difference between the body weight gains of the two groups. This could be attributed to the feed intake. In the case of the feed intake, there was positive increase in each group, the feed intake increased from 715 g to 835 g and from 730 g to 960 g for group A and group B respectively. The difference may be due to the nutrient content of defatted *G. mangostana*. The feed intake obtained in this study was a little bit higher than 625g reported by Ajayi *et al.* (2007) on rats fed with *Garcinia mangostana* seed oil. The result is also similar to the report given by (Longvah *et al.*, 2000) for rat fed with groundnut and Perilla oil.

ii. Organ Weight Changes

Table 6 shows the organ weights of test and control rats for six weeks experiment. The organs whose weights were noted were liver, kidney, heart, lungs, intestine, brain and spleen. The liver weight obtained in groups A and B (4.25 ± 0.68 and 4.01 ± 0.37) respectively is similar to the report given by Vishnu *et al.* (2010) where the control group was 4.1 ± 0.26 and the test group ranged from 3.94 ± 0.94 to 4.01 ± 0.31 . Vishnu *et al.* (2010) found also no significant difference between the control group and various dose of *G. mangostana* pericarp applied on rat. The values of heart, brain, and lungs: 0.5 ± 0.00 , 1.5 ± 0.00 and 0.92 ± 0.37 respectively obtained in group A are similar to the weight in group B.

g) Haematological Analysis Results

Table 7 shows the result of haematological analysis of *G. mangostana* seed cake on rats. The parameters obtained for rats in group A and group B were comparable. Haemoglobin concentration in group B (14.35 ± 0.35) is significantly different to that of Group A (13.88 ± 0.62). Bienat *et al.* (1997) obtained 13.88 ± 0.1 for diet without cholesterol addition of *Oenothera paradoxa* seed oil. Vishnu *et al.* (2010) obtained a concentration ranging from 13.83 ± 0.98 to 14.12 ± 0.41 for *G. mangostana* pericarp extracts in rats at 1g/kg, 2g/kg, and 3g/kg respectively. The MCV concentration in groups A and B are very comparable (60.32 ± 1.40 and 5907 ± 0.81). WBC in group B was 4750 ± 1240 which is lower to that in group A, 5591.67 ± 2218 . A high value ranging from 8050 ± 330.9 to 8633 ± 513.3 was obtained by Ajayi *et al.* (2007). Since the haematological parameters obtained from rats fed with *Garcinia mangostana* seed cake compared favourably with the values obtained with the rats fed with normal feed (control group), it indicates that GMSC has no adverse effect on the blood of the test rats.

h) Histopathological Result

The pathology result showed no major complications and no significant differences on the tissues of the rats in both groups (Table 8).

IV. CONCLUSION

Based on the proximate analysis results obtained from this study, *Garcinia mangostana* seed cake showed a high value for carbohydrate content but it is low in crude protein and crude fibre. It can be utilized for roughage in feed for live stock because of the high value of carbohydrate and can be supplemented with other high protein residue, such as groundnut cake. The phytochemical analyses showed the presence of tannins, flavonoids and alkaloids which ascertain that *Garcinia mangostana* seed cake contains some major compounds that have remarkable biological activities, and might be helpful in preventing various diseases. Defatted *G. mangostana* contains Fe, Zn, Mg, Na, Cu, K, Mn and Ca, among which Fe and Mn concentration are high but Fe has the highest value. These metals are all useful in making the body strong. *Garcinia mangostana* seed cake did not produce any significant change of haematological parameters as well as in the heart of the rats in both groups. The seed cake might be a good additive in feed supplement.

V. ACKNOWLEDGEMENTS

The authors thank the Department of Chemistry, Faculty of Science and Department of Veterinary Pathology, Faculty of Veterinary of University of Ibadan, Ibadan, Nigeria for making their facilities available.

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