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ScienceDirect
Food Chemistry 101 (2007) 999–1004
Food Chemistry
www.elsevier.com/locate/foodchem

Chemical analysis and preliminary toxicological evaluation of *Garcinia mangostana* seeds and seed oil

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Received 22 September 2005; received in revised form 23 February 2006; accepted 23 February 2006

Abstract

The chemical analysis and preliminary toxicological evaluation of *Garcinia mangostana* seeds and seed oil have been investigated in order to determine the possibility of using them for human and/or animal consumption. Proximate analysis showed that the seeds had high amount of carbohydrate and were rich in oil ($21.68 \pm 6.18\%$) but have a low protein content. The physical properties of the oil extracts showed the state to be liquid at room temperature (25 ± 1 °C) and the colour of the oil golden-orange. The specific gravity of the oil was 0.98 ± 0.01. Among the chemical properties of the oil extracts, acid value, saponification number, iodine value, percent free fatty acid and peroxide value compared well with those of conventional edible oils. The seed flour was found to be a good source of minerals. It contained considerable amounts of potassium (7071 mg/kg), magnesium (865 mg/kg) and calcium (454 mg/kg). Fatty acid composition of the seed oil indicated that the oil contained one essential fatty acids small proportions: linoleic acid (1.30%). The most prevalent fatty acids were palmitic acid (49.5%) and oleic acid (34.0%). Weanling albino rats appeared to suffer no toxicological effects when fed with *G. mangostana* seed oil in their diet for 8 weeks. Weekly monitoring of the rats showed good physical appearance and steady weight increase. Histological examination of sections of the heart, liver, kidney, spleen and lung revealed that the kidney of some of the rats had some degrees of pathology which included diffuse glomerular and tubular degeneration. No lesion was found in the heart and liver of the rats. The seed oil could be useful as an edible oil and for industrial applications.

Keywords: *Garcinia mangostana*; Mineral elements; Fatty acid; Toxicology effect

1. Introduction

Conventional edible oils are becoming very scarce and there is a need to establish alternative oil-bearing seeds as their substitutes (Agbaji, Terry, & Agbaji, 1993). There are hundreds of species of trees which provide food for people in both the humid and semi-arid tropics, but they have received much less attention from the scientific community than the annual crops (Cannel, 1989). Preliminary compositional studies carried out on seeds of *Garcinia mangostana* showed that they deserve to be investigated as promising sources of fat and carbohydrate for possible use as food/feed to bridge the gap of oil deficiency. Recently, more attention has been focussed on the utilization of food processing by-products and waste, as well as under-utilized agricultural products. Obviously, such utilization would contribute to maximizing available resources and result in the production of various new products and thereby avoid waste disposal problems. The continued increase in world population and the ever-increasing demand for both oils and oilmeal have resulted in increase in the prices of oils. This increase in prices necessitates the need to investigate new sources of oils, especially among the non-conventional and under-exploited oil-seeds (Omode, Fatoki, & Olaogun, 1995). The search for alternative oil sources, especially for developing countries, is of utmost importance. There already exist abundant data on

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0308-8146/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2006.02.053

the proximate composition, mineral content and other characteristics of the more conventional oil seed types (Oyenuga, 1968) but not on the non-conventional ones such as *G. mangostana*.

G. mangostana, (Mangosteen) a *Guttiferae*, is one of the most widely recognized tropical fruits and has universal appeal because of its quality in colour, shape and flavour. Mangosteen, known as the 'Queen of fruits', originated from southeast Asia, probably Malaysia, but it can now be found in several tropical countries. The white, moist, soft and juicy flesh is sweet and has a high sugar content (Kanchanapoom & Kanchanapoom, 1998; Martin, 1980; Nakasone & Paul, 1998). The pulp has an excellent flavour and, though slightly acidic, it is sweet and delicious. *G. mangostana* has some medicinal properties. It possesses anti-inflammatory, astringent, antibacterial, antitumor and antioxidative activities (Chairrungsri, Takeuchi, Ohizumi, Nazoe, & Ohta, 1996). Ethanolic extracts of selected Thai medicinal plants tested for anti-proliferate activity against SKBR3 human breast adenocarcinoms cell line using MTT assay, revealed that *G. mangostana* had the most potent activity (Moongkarndi, Kosem, Lurantana, Jogsonboonkusol, & Pongpan, 2004).

In Nigeria, there is little or no information on *G. mangostana*. The seed is neither eaten nor used for any industrial purposes. The aim of this work, therefore, is to analyze the chemical composition of *G. mangostana* seed and its oil and to achieve preliminary toxicological evaluation of the oil and understanding of its food chemistry.

2. Materials and methods

2.1. Plant material

Garcinia mangostana fruits were obtained from the Botanical Garden of the University of Ibadan. The seeds were removed from the fruits, washed with water and left to air-dry for two days.

2.2. Sample preparation

The seeds of *G. mangostana* were decorticated manually, and ground into a paste using a previously-cleaned and dried mortar and pestle. The paste was then stored in an air-tight container in a refrigerator (4 °C) prior to analysis.

2.3. Proximate analysis

The moisture content of the seed was determined gravimetrically by placing 1 g of the sample in an oven at 102 °C for 6 h to reach constant weight (Femenia, Rosells, Mullet, & Canellas, 1995). The seed oil was extracted using the continuous Soxhlet solvent extraction technique with a good grade petroleum ether as solvent (boiling point range 40–60 °C) for 8 h (Oderinde & Ajayi, 1998). Nitrogen content was estimated by the Kjeldhal method AOAC (1984) and crude protein was calculated ($N \times 6.25$). Crude fibre and ash were determined in accordance with the standard methods of the AOAC (1980). The value for the carbohydrate content was obtained by computation (Al-Khalifa, 1996).

2.4. Physical properties

Oil from the seed was subjected to physical characterization. The colours and state of the oil at room temperature were noted by visual inspection, while density was determined by the method of the AOAC (1980). The refractive index of the oil at room temperature was estimated using the Abbe refractometer as outlined by Pearson (1982) and Ajayi et al. (2002).

2.5. Chemical composition

Procedures for the determination of acid and peroxide values were as outlined by Ajayi and Oderinde (2002). The analyses for iodine value (Wijs' method) and saponification number were carried out following the official method (AOAC, 1984). The estimation of the percentage free fatty acids as oleic acid was done, following the method described by Cock and Rede (1966).

2.6. Analysis of mineral elements

The wet-ashing method was employed for the digestion of the seed sample; 1 g of defatted *G. mangostana* seed was digested with 20 ml of concentrated HNO_3 and perchloric acid (1:1 v/v) and thereafter transferred to a 50 ml volumetric flask. It was diluted to volume 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).

2.7. Fatty acid analysis

The analysis of fatty acids in the seed oil was carried out at the Institute of Organic Chemistry, University of Tuebingen, Germany, following the method described by Ajayi, Adebowale, Dawodu, and Oderinde (2004). The fatty acid methyl esters were prepared by adding 5 ml of CH_3OH and 1 ml CH_2Cl_2 to 0.1 g of the oil. The mixture was cooled in ice and 0.6 ml of CH_3COCl was added; 1 ml of the solution was withdrawn into the hydrolysis tube and heated for 1 h at 110 °C. The solution was cooled and discharged into 10 ml of 1.00% NaC1 solution in a separating funnel. The organics were extracted with 3×4 ml hexane and the volume was reduced to 0.5 ml using a rotatory evaporator. This was eluted on silica gel column successively with 5 ml hexane and 4 ml CH_2Cl_2. The CH_2Cl_2 fraction was separated on a DB5 30 m × 0.25 mm capillary installed on a GC Chrompack 9001 equipped with computer software and mosaic integration. A flame ionization detector was used. The temperature was programmed as follows: 35 °C

for 3 min, then the temperature was increased at 20 °C per minute up to 230 °C for 5 min. Heptadecanoic acid was used as an internal standard.

2.8. Animals, diets and feeding

Fifteen weanling albino rats (aged 4 weeks, weighing between 50 and 70 g) were obtained from the University of Ibadan, Nigeria. The animals were divided into three groups of five rats per group and were housed for a period of 8 weeks before sacrifice, during which time they were allowed access ad libitum to water and a commercial rat feed (Ladokun Feeds Limited, Ibadan, Nigeria). At the commencement of the experiment, the control group (group 1) were fed with the commercial rat feed only; group 2 rats were fed with commercial rat feed mixed with 5% groundnut oil; while the group 3 rats were fed with the commercial rat feed mixed with 5% *G. mangostana* oil. The body weight of each rat was recorded weekly for the 8 weeks of the experiment. Animals were sacrificed after a 14–16 h overnight fast on the last day of the experiment.

2.9. Haematological examination

For haemotological analysis, 3 ml of blood were collected by cardiac puncture into heparinized vials and stored at 10 °C for analysis the same day. 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).

2.10. Organ/tissue pathology

The abdominal wall was dissected through the linear alba and peritoneum using a scalped blade. The liver, heart, kidney, spleen and lung of each rat were examined for gross lesions. A 0.5 cm^3 sample of each organ was fixed in 10% phosphate-buffered formalin and prepared for histological examination, following the method of Raghuramulu, Nair, and Kalyanasundaram (1983). Different sections of each organ were examined for lesions using an Ortholux light microscope (Leitz-Weiltzer, Germany GmBh).

2.11. Haematological examination

The packed cell volume and white blood cell count were determined using the standard technique described by Dacie and Lewis (1991), and Jain (1986). The haemoglobin concentration and erythrocytes count were also estimated. Parasitic examination of the blood sample was also carried out. A thin smear of uncoagulated blood was made on a labelled, cleaned, greased slide. The smear was air-dried and then fixed by flushing with methanol for 3 min. The fixed smear was then rinsed with buffer solution and stained with Giemsa for 45 min. Observation at 100 × objective, after a drop of oil immersion, was done to check for the presence or absence of intra- or extra-erythrocytic haemoprotozoan parasites.

2.12. Statistical analysis

Results are expressed as the means and standard errors of three separate contents, except for mineral elements and fatty acid. The data were statistically analyzed by (SAS, 1987) 2-way analysis of variance (ANOVA). Means were compared by Duncan's multiple range test (Duncan, 1955) at 5% level of significance ($P \leqslant 0.05$).

3. Results and discussion

3.1. Proximate analysis

The results of the proximate composition of *G. mangostana* are shown in Table 1. The oil yield of the seed, 21.18 ± 6.18 g/100 g is closely similar to those reported for various soybean cultivars, 18.30–21.53 g/100 g dry matter (Vasconcelos et al., 1997). It also compares favourably with 21.0% of *C. lanatus* (Al-Khalifa, 1996) and *M. myristica* (Ajayi et al., 2004). The protein content of the seed is quite low, but much higher than the 5.29 ± 0.28 g/100 g reported for *C. tuberos* (Oderinde, Tairu, Dawodu, & Bamiro, 1990) and slightly higher than the values for crude fibre of corn (Heger & Eggum, 1991). The crude fibre content, 13.7 ± 0.89 g/100 g and carbohydrate, 43.5 g/100 g indicate that the seeds are good sources of these two parameters and suggest that they could serve as source of roughage in animal feeds. The ash content, 1.99 g/100 g, is greater than the values determined for seeds such as coconut, kolanut and melon but less than those of castor, groundnut and oil bean seeds (Onyeike & Acheru, 2002).

3.2. Physical and chemical properties

The oil extract, which is consistently liquid at room temperature (25.0 ± 2.0 °C), has a golden-orange colour (Table 2). The specific gravity and refractive index of the oil are 0.98 ± 0.01 and 1.482, respectively. The value for

Table 1
Proximate composition of *Garcinia mangostana* and groundnut seeds (g/100 g)[a]

Constituents	*Garcinia mangostana*[b]	Groundnut seeds[c]
Moisture	13.08 ± 1.99	4.45 ± 0.32
Ash	1.99 ± 0.30	2.77 ± 0.65
Crude protein	6.57	26.5 ± 0.27
Crude fat	21.18 ± 6.18	40.8 ± 0.50
Crude fibre	13.7 ± 0.89	25.4 ± 0.59
Carbohydrate	43.5 ± 2.09	–

[a] Values are means ± standard deviation of triplicate determinations.
[b] Present work.
[c] Onyeike and Acheru (2002).

Table 2
Physical and chemical properties[a] of oil extracts from *Garcinia mangostana* and groundnut seeds

Component	*Garcinia mangostana* seed[b]	Groundnut seeds[c]
Acid value (mg NaOH/g oil)	4.58 ± 0.16	2.77 ± 0.71
Saponification number (mgKOH/g oil)	134 ± 2.14	362 ± 2.78
Iodine value (mg/ lOOg)	53.64 ± 0.15	11.2 ± 1.73
FFA (%) as oleic acid[d]	2.31 ± 0.08	0.44 ± 0.14
Peroxide value (mg/g oil)	3.27 ± 0.12	20.0 ± 2.10
Ester value (mg/KOH)	130 ± 2.14	–
State at RT[d]	Liquid	Liquid
Colour	Golden-orange	Pale yellow
Specific gravity	0.98 ± 0.01	0.89
Refractive index at RT	1.482	

[a] Values are means ± standard deviation of triplicate determinations.
[b] Present work.
[c] Onyeike and Acheru (2002).
[d] FFA (%) = free fatty acid (%).

the refractive index of the oil is slightly higher than that of *P. macrophylla*, 1.4696 (Ajayi, Dawodu, Adebowale, & Oderinde, 2002). Some chemical properties of the oil extract of the seed analyzed are presented in Table 3. The total acidity, expressed as acid value, is 4.58 ± 0.16 mg NaOH/g. It compares favourably with values for sesame, soybean, sunflower and rape acid, 2.31 ± 0.08 mg KOH/g and is similar to the values of 2.82 ± 0.14 mg and 2.815 ± 0.135 mg reported for the pulp and seed of *D. edulis*, respectively (Ajayi & Oderinde, 2002). These values are within the allowable limits for edible oils (Eckey, 1954). The nutritional value of a fat/oil depends, in some respects, on the amount of free fatty acids (e.g. butyric acid in butter) which develops.

In the tropics, where vegetable oils are the most common dietary lipids, it has been shown that it is desirable to ensure that the free fatty acid contents of cooking oils lie within limits of 0.0–3.0% (Bassir, 1971). The low level of FFA in the oil *G. mangostana* suggests that the oil could be a good edible oil that will store for a long time without spoilage via oxidative rancidity. The low free fatty acid values of *Chryosophyllum albidum* (1.81 ± 0.1) and *Cola rostata* (5.0 ± 020) seed oils have been reported to support the view that these oils are edible oils and could have long shelf lives (Dosunmu & Ochu, 1995). The peroxide value of the oil is 3.27 ± 0.12 mg/g oil, suggesting that it can be stored for a long period without deterioration. According to Ojeh (1981), oils with high peroxide values are unstable and easily become rancid (having a disagreeable odour). Pearson (1982) also reported that fresh oils have been shown to have peroxide values below 10 mg/g oil and oils become rancid when the peroxide value ranges from 20.0 to 40.0 mg/g oil. The saponification number of the *G. mangostana* oil is low (134 ± 2.14 mgKOH/g); hence it is not likely to be suitable for soap making. The iodine value of the oil, 53.6 ± 0.15 mg/100 g, places it in the non-drying group of oils. The Codex Alimentarius Commission (1982) stipulated a permitted maximum peroxide level of not more than 10 mg peroxide oxygen/kg oil, the peroxide value of the oil from *G. mangostana* seeds is well below 10; hence it may be suitable as an edible oil.

Table 3
Mineral element contents of *Garcinia mangostana* seeds (mg/kg of dry matter)

Mineral element	*Garcinia mangostana* seeds
Potassium	707
Calcium	454
Sodium	26.0
Magnesium	865
Zinc	19.0
Iron	90.0
Copper	ND[a]
Manganese	18.0

[a] ND = not detectable.

3.3. Mineral elements

The human body requires a number of minerals in order to maintain good health. A number of minerals essential to human nutrition are accumulated in different parts of plants (Dushenkov, Kumar, Motto, & Raskin, 1995). Plants are known to supply the needed vitamins, iron, calcium, magnesium and others important for human health and they are the most affordable source of minerals and vitamins for African families (Anne, 1979; Schutlink, West, & Pepping, 1987). The results for the mineral element composition of *G. mangostana* seeds (Table 4) show that the seeds have a high level of potassium, 7071 mg/kg, followed by magnesium, 8650 mg/kg and calcium, 454 mg/kg. Potassium is an essential mineral element which helps to regulate blood pressure, while calcium is needed for bone growth and muscle contraction and in blood clotting. Magnesium works with calcium to maintain healthy bones. Calcium is also very important in the maintenance of a healthy heart. A diet containing *G. mangostana* seeds will help prevent deficiency of potassium, magnesium and calcium since the seeds are rich in these elements. Other elements present in the seeds are

Table 4
Fatty acids composition of *Garcinia mangostana* and groundnut seed oils

Fatty acid	*Garcinia mangostana* oil[a]	Groundnut oil[b]
C_{16}:0 Palmitic	49.5	12.6
C_{18}:0 Stearic	1.33	1.8
C_{16}: 1 Palmitoleic	ND	1.2
C_{18}:1 Oleic	34.2	47.8
C_{18}:2 Linoleic	1.03	30.2
C_{18}:3 Linolenic	ND	ND
C_{20}:0 Arachidic	8.77	4.2
C_{20}:1 Gadoleic	0.10	ND
C_{20}:2 Eicosadienoic	0.11	ND
C_{22}:0 Behenic	ND	1.9
C_{24}:0 Lingnoceric	ND	0.3
Unknown	5.14	ND
Total saturates	59.6	20.8
Total unsaturates	35.3	79.2

[a] Present work.
[b] Longvah et al. (2000).
[c] ND : Not detected.

manganese, iron and zinc. Copper was not detectable in the seed. The iron content of *G. mangostana* seed, 90.0 mg/kg, is higher than those of *Cicer arietinum*, 60.0 mg/kg, *Phaseobus mungo*, 41.0 mg.kg and *P. aureus* 30.0 mg/kg.

3.4. Fatty acids

The fatty acid composition of an oil is its most useful chemical feature. Many of the chemical tests for oil identity or purity can be related to their fatty acid content (Pritchard, 1991). Table 5 shows the analysis of *G. mangostana* seed oil. The most prevalent unsaturated fatty acid is oleic acid (34.0%). The oil contains two out of the three essential fatty acids, namely linoleic and arachidic acids. The fatty acid composition of the oil indicates that it contains a high proportion of palmitic acid (49.5%). The total saturated fatty acid is 59.6% while the total unsaturated fatty acid is 35.3%; 5.14% represents the percentage of the unknown fatty acids in the seed oil.

3.5. Feed intake and body weight changes

The feed intake and the resultant body weight changes of test and control rats are shown in Table 6. There is no significant difference between the feed intakes of the rats from the test and control groups ($P > 0.05$).

Rats from the test group displayed fairly similar body weight gain to those from the normal control group as there was no significant difference between the body weight the gains of the different groups ($P > 0.05$). This observation is similar to that in the report given by Longvah, Deosthale, and Kumar (2000) for rats fed with groundnut oil and Perilla seed oil, and that of Perez-Granados, Vaguero, and Navaro (2000) for rats fed on olive and sunflower oils.

3.6. Haematological parameters

The haematological parameters and indices obtained for rats fed with *Garcinia mangostana* seed oil compare favourably with the values obtained for rats fed with the normal feed (control I) and groundnut oil (control II). This indicates that the oil from *G. mangostona* had no adverse effects on the blood of test rats. The haematological values obtained from rats in this study are similar to those reported for healthy rats and related murine species (Ogunsanmi, Ozegbe, Ogunjobi, Taiwo, & Adu, 2002; Oyewale, Olayemi, & Oke, 1998).

Table 5
Body weight changes and feed intake (g) of test rats and control I and II rats

Week Body weight changes	Test rats	Control I rats	Control II rats
1	75 ± 7	84 ± 6^{b}	71 ± 76^{b}
2	89 ± 9	99 ± 2^{b}	80 ± 4^{c}
3	85 ± 9^{b}	123 ± 5^{c}	101 ± 13^{b}
4	106 ± 7^{b}	135 ± 9^{c}	11 ± 9^{b}
5	130 ± 14^{b}	150 ± 10^{b}	130 ± 16^{b}
6	146 ± 17^{b}	172 ± 11^{b}	138 ± 27^{b}
7	150 ± 10^{b}	180 ± 14^{c}	141 ± 18^{b}
8	152 ± 14^{b}	180 ± 20^{b}	158 ± 21^{b}
Feed intake	600 ± 40^{b}	600 ± 20^{b}	625 ± 85^{b}

Means ± standard deviation, $n = 5$.
Means in the same row having the same letters are not significantly different at the 5% level.

Table 6
Result of haemotological analysis

Parameter	Test rats	Control I	Control II
PCV%	49.00 ± 4.00^{b}	48.40 ± 4.62^{b}	47.80 ± 5.81^{b}
RBC count (10^6/ul)	7.12 ± 0.66^{b}	6.03 ± 0.36^{b}	6.07 ± 0.95^{b}
Hb (mg/dl)	15.7 ± 1.81^{b}	15.9 ± 1.68^{b}	15.3 ± 1.62^{b}
MCV (fl)	69.0 ± 1.77^{b}	80.3 ± 5.85^{b}	79.0 ± 2.82^{b}
MCHC (%)	32.0 ± 1.20^{b}	32.8 ± 1.06^{b}	32.0 ± 0.92^{b}
WBC count (10^3/µl)	4.84 ± 1.43^{b}	4.89 ± 0.79^{b}	5.80 ± 1.62^{b}
Lymphocyte	3.88 ± 0.81^{b}	3.88 ± 0.69^{b}	4.18 ± 1.33^{b}
Neutrophis	0.85 ± 0.42^{b}	0.78 ± 0.17^{b}	1.28 ± 0.37^{b}
Eosmophis	0.08 ± 0.07^{b}	0.03 ± 0.03^{b}	0.12 ± 0.23^{b}
Monocytes	0.17 ± 0.07^{b}	0.20 ± 0.10^{b}	0.22 ± 0.06^{b}

Means ± standard deviation, $n = 3$.
Means in the same row having the same letters are not significantly different at the 5% level.

3.7. Histopathology

No mortality was recorded in any of the control and test rats throughout the duration of study. No lesions were observed in the organs of the control (group I) rats, except for one which had slightly depopulated splenic white pulp and another with congested cardiac blood vessels. However, rats fed with *G. mangostana* oil in their diet had mild cortical congestion, locally diffuse glomerular and proximal tubular degeneration and presence of pink-staining proteinacean casts in the tubular lumen. There was also mild cortical fibrosis and interstitial lymphocytic infiltration in the medulla. Similar, but yet milder lesions were observed in the kidneys of rats fed with 5% groundnut oil in the diet.

No lesions were observed in the liver, spleen or heart of rats fed with *G. mangostana* oil and groundnut oil. These findings indicate that the oil from *G. mangostana* is not harmful to most organs and tissues of rats at 5% inclusion level. Hence, it can be used to replace groundnut oil or any other similar conventional oils in the diet of livestock and even man. Lower levels, that is <5%, are however, recommended to avoid kidney damage.

4. Conclusion

Garcinia mangostana seeds could be utilized successfully as sources of dietary fibre and for roughage in feed for livestock because of their high crude fibre and carbohydrate contents. The protein content, which is low, can be supplemented with other high protein residues, such as groundnut or soy cakes. The physicochemical properties of *G. mangostana* oil compare favourably with those of conventional edible oils; percent free fatty acids and peroxide value are

below the maximum desirable limit and this suggests the suitability of the oil as an edible oil.

The seed oil, when fed to rats, was found not be toxic to the liver, heart or spleen of the rats and none of the rats died throughout the period of the experiment. The lesions observed in the kidney of the rats were quite mild and not peculiar to the test rats alone, as similar lesions were found in rats given groundnut oil in their diet.

It can thus be concluded that the oil of *G. mangostana* has no deleterious effects on rats, but could be administered at <5% inclusion level in order to avoid possible kidney damage.

Acknowledgements

The authors thank the Departments of Chemistry, Botany and Veterinary Pathology, University of Ibadan, Nigeria and the University of Tuebingen, Germany for making their facilities available for use during the study.

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