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Nutritional Composition and Antimicrobial Activity of Fractionated Extracts of *Garcinia kola* Heckel

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Abstract. Seeds of *Garcinia kola* were analysed for mineral and amino acid composition. Solvent extraction and fractionation of bioactive components of the seeds were accomplished using Soxhlet extractor and vacuum liquid chromatography, respectively. Fractionated extracts were assessed for sensory and antimicrobial activities. The seed contained varying amounts of micro nutrients with low amounts of anti-nutritional elements. Amino acids found at considerable quantities in the seed of *Garcinia kola* were essential amino acids like L-lysine, L-histidine, L-arginine, L-valine, L-methionine, L-isoleucine, L-leucine and L-phenyl alanine. Fractionation of the dark brown - bitter crude extract yielded some fractions (F_4, F_5) that were yellow in colour with non-bitter taste. The fractionated extracts exhibited antimicrobial activities. The most potent fraction (F_4) had minimum inhibitory concentrations of 1 μg/ml on each of the organisms, *Bacillus cereus*, *Streptococcus faecalis*, *Candida albicans* and minimum inhibitory concentrations (μg/ml) of 0.125, 0.5, 2 on *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, respectively. The fractionated extracts can be exploited to preserve food systems from undesirable activities of microorganisms.

Keywords: *Garcinia kola* seeds, nutrients, fractionated extracts, sensory attributes, microbial inhibition

Introduction

Nature's nutritional enrichment of seeds suggest that the seed of *Garcinia kola* is endowed with some food values. Oyenuga (1968) reported proximate composition of the pulp and the rind of *Garcinia kola*. Essien *et al.* (1995) reported the lipid composition and fatty acid profile of the pulp and rind of *Garcinia kola*. However, to our knowledge, detailed minerals and amino acid composition of *G. kola* seed grown in Nigeria have not appeared in literature to date.

In addition, exploitation of protective endowment of seed for treatment of human and animal diseases stretches back into antiquity. Seed of *G. kola* is used in Nigerian traditional medicine for therapy of a broad spectrum of ailments including dysentery and diarrhoea. Besides, *G. kola* is used in treatment of cough, jaundice, snake bite and other forms of poisoning (Adeniji, 2003). The seed has been reported to contain secondary metabolites principally biflavonoids with antihepatoxic action (Iwu, 1985). Sodipo *et al.* (1991) also reported pharmacological and antimicrobial activities of the *G. kola* seed.

The pharmacological and antimicrobial properties of *G. kola* suggest that bioactive components of the seed of *G. kola* may find applications in prolonging shelf life of food products. However, bitter components of the seed will be a major drawback to utilization of the natural preservative in food, hence should be eliminated. Therefore, dearth of information on detailed mineral and amino acid composition and need for elimination of bitterness in crude extracts of *G. kola* serve as impetus for this study.

The objectives of this study were: determination of mineral and amino acid composition of *G. kola* seed, fractionation of crude extract, assessment of organoleptic and antimicrobial activity of fractionated extracts.

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Materials and Methods

Source of materials. Seeds of *G. kola* used in this study were obtained from fresh ripen fruits harvested from the parent plant within a Cocoa plantation in Ikere-Ekiti, Ekiti State, Nigeria. The seeds were dried at a temperature of 50 °C for 5 days in air oven, pulverized and packaged for subsequent analysis. All the culture media used were commercial products. The agar used was a product of Agar technical (Oxoid), nutrient broth used was the product of Biotech. International and Sabourand dextrose agar used was manufactured by International Diagnostic Group Plc., Lascashire BL 96 Au, UK. These culture media were prepared according to the manufacturer's instructions.

Determination of nutritional, proximate and mineral composition. Proximate composition of the samples were determined according to standard methods (AOAC, 1990) with Analytical Codex Number 14.062, 14.063, 14.064, 14.066 and 14.067 for

moisture, total ash, crude fat, crude fibre and crude protein, respectively. Moisture and fat contents were determined gravimetrically by oven drying and Soxhlet extraction methods, respectively. Defatted samples were treated with standard solvents in order to estimate the amounts of crude fibre. Amounts of crude protein were determined using a Tecator Nitrogen Analyser System. Ash contents were determined by heating samples in Muffle furnace. Amounts of carbohydrate were obtained by difference. All minerals, with the exception of phosphorus, were evaluated using the method of AOAC (1990). Samples were analysed using Alpha-4 (Chemtech Analytical) atomic absorption spectrophotometer. Total phosphorus content was appraised using vanadomolybdate yellow colour method (AOAC, 1990).

Amino acid determination. Amino acid profile of the seed was determined using amino acid analyzer (Moore *et al.*, 1958; Spackman *et al.*, 1958). Samples were defatted and hydrolysed at 15 psi for 17 h in sealed glass tubes containing 3 M HCl. The hydrolysates were recovered in 0.2 M sodium citrate buffer at pH 2.2. The amino acids in the hydrolysates were analysed by using amino acid autoanalyzer model JEOL-6-AN with the columns packed with LCR-2 spherical resin at 52 °C. At the short column, the buffers were at pH of 5.28 and a flow rate of 1.15 cm^3/min and at the long column, the buffers used were at pH of 3.00, 3.50 and 4.25 with a flow rate of 0.84 cm^3/min. Peaks were identified by comparing with elution times of standard samples. Analyses were done in duplicate.

Solvent extraction of bioactive components (crude extract) and crude extract of seeds of *G. kola*. Extraction of bioactive components of the seeds was done by using ethanol and diethylether in Soxhlet extractor, based on the method described by Adegoke and Krishna (1998).

Fractionation of crude extracts using vacuum liquid chromatography (VLC). Fractionation of ethanolic crude extracts was accomplished using VLC in accordance with the method of Odukoya *et al.* (1999).

Assessment of sensory characteristics of crude and fractionated extracts of the seed of *G. kola*. Sensory characteristics of crude and fractionated extracts of the seed were determined subjectively (Demooy and Demooy, 1990).

Determination of antimicrobial activities of crude and fractionated extracts of the seed *G. kola* using cup-plate method and turbidity procedure. Antimicrobial potentials of crude and fractionated extracts of the seeds in some microorganisms, type or standard of which was evaluated. Assessment of antimicrobial activity was accomplished following essentially the procedure elicited in Adegoke (2004). The antimicrobial activity was assessed by determining the zone of inhibition and minimum inhibitory concentrations (MICs) of the test extracts using cup-plate or agar diffusion method and turbidity procedure, respectively on test organisms. Each of the organisms was subjected to the crude and fractionated extracts of the seed of *G. kola* at recommended concentration of 0.02% for cup-plate method and at concentrations (μg/ml) of 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.031 for turbidity method. Microorganisms used in assessment of antimicrobial activities of the crude and fractionated extracts of the seed *G. kola* were: *Bacillus cereus* (NCIB 6349); *Klebsiella pneumoniae* (NCIB 418); *Streptococcus faecalis* (NCIB 775); *Escherichia coli* type (1) (NCIB 86); *Staphylococcus aureus* (NCIB 8588) and the fungus *Candida albicans*.

Results and Discussion

Nutritional composition of the seeds. The proximate composition (%) of *G. kola* seeds is shown in Table 1. The elements in the seeds evaluated in this study are from both major (elements required in amount greater than 100 mg/day) and trace groups (element required in amount less than 100 mg/day).

Previous report showed that mineral analysis was evaluated on the fruit pericarp and mesocarp (Osagie and Eka, 1998).

Table 1. Nutrient composition of *Garcinia kola* seeds

Nutrient	Means ± SEM[b]
[a]*Proximate composition*	
Moisture	6.30±0.02
Crude fat	1.70±0.00
Crude protein	3.49±0.05
Total ash	2.00±0.10
Crude fibre	2.55±0.08
[c]Carbohydrate	83.96±0.00
[d]*Mineral composition*	
Iron	60.21±0.04
Copper	1.04±0.00
Zinc	11.92±0.07
Manganese	8.84±0.012
Calcium	72.06±0.03
Magnesium	193.64±0.69
Sodium	31.02±0.21
Potassium	720.00±2.1
Phosphorus	1090.00±0.00
Arsenic	3.05±0.00
Lead	0.13±0.00
Cadmium	[e]ND
Cobalt	0.60±0.00

[a] = % dry sample, [b] = standard error of mean, [c] = obtained by difference, [d] = mg/100 g dry sample, [a,d] = all data are average of two determinations, [e] = not detected

However, there is no literature report on the mineral profile of the seeds of *G. kola*. The results (Table 1) obtained in this study showed that calcium and zinc contents of the seed of *G. kola* compared favourably with the garlic (Zn = 3 mg/100 g; Ca = 80 mg/100 g) and cardamon (Zn = 7 mg/100 g; Ca = 383 mg/100 g). The magnesium level of the seed is higher (193.64 mg/100 g) than the garlic (58 mg/100 g) and comparable with the cardamom (Farrel, 1985). The seeds of *G. kola* is very rich in phosphorus (1.090 g/100 g), a mineral required for many biological functions (Smith and Ojofeitimi, 1995). The dietary iron (60.2 mg/100 g) found in the seed shows that utilization of 50-100 g of *G. kola* seeds can help to meet the daily requirement of 30-60 mg of dietary iron required for pregnancy or lactation (National Research Council, 1980). These seeds contain low amounts of assessed antinutritional elements.

Amino acid profile of the seeds. In addition to the array of minerals present in the seeds of *G. kola*, it also contains both essential and non-essential amino acids (Table 2). The essential amino acids found in the seeds of *G. kola* compares quantitatively with those reported for cow milk (Heine *et al.*, 1991). However, protein of the *G. kola* is relatively deficient in sulphur containing amino acids (methionine and cysteine). Nutritional significance of protein and amino acids are well documented in literature (Smith and Ojofeitimi, 1995). Essential amino acids cannot be made by the body and must be supplied in the diet. All but L-arginine and L-histidine are necessary for adult and for growing children. Consequently, the amino acid in the seeds of *G. kola* will play a significant role in securing a balanced diet on consumption.

Sensory characteristic of crude and fractionated extracts of the seeds. The active components of the seeds of *G. kola* were separated into six fractions using vacuum liquid chromatography (VLC), their sensory characteristics in relation to crude extracts are shown in Table 3. The crude extracts, namely ethanolic extract (ETH) and diethylether extract (DDE) were dark brown viscous liquid with lingering bitter taste. The dominating bitter taste is as a result of alkaloids present in *G. kola* (Evans *et al.*, 2002; Braide, 1986). The ethanolic fractionated extracts (F_1, F_2, F_3, F_4, F_5, F_6) obtained from the VLC technique were of varying degree of yellowish colour with variable taste profile (Table 3), which could have been as a result of the dominant bioactive components (alkaloids, flavonoids, anthraquinones) as revealed by phytochemical screen test (Result not shown).

Interestingly, some fractions (F_4 and F_5) obtained by using VLC were light yellow and sun-set yellow in colour, respectively, with a non-bitter taste in contrast to the dark colour and bitter taste associated with the crude extracts (ETH, DDE) of the seeds of *G. kola*. Such fractionated extracts will be a good candidate for preservation of food systems against microbial deterioration and its undesirable consequences, if it possesses antimicrobial -proliferation potentials. Consequently, the fractionated extracts (F_4, F_5) were examined for antimicrobial activity in relation to the crude extracts (ETH, DDE).

Antimicrobial activity of crude and fractionated extracts of the seeds of *G. kola*. The crude extracts and fractionated (F_4 and F_5) extracts of the seeds exhibited inhibitory activities on some microorganism namely; *Bacillus cereus*, *Escherichia*

Table 2. Amino acid profile of the seed of *G. kola*

Amino acid	[a]Amount (g/100 g)		
	G. kola seed	Seed protein	[b]Cow milk protein
L-Lysine	0.2554	7.310	7.60
L-Histidine	0.0874	2.500	2.60
L-Arginine	0.3934	11.270	-
L-Aspartic acid	0.2705	7.750	-
L-Threonine	0.1434	4.110	4.60
L-Serine	0.1175	3.370	-
L-Glutamic acid	0.7089	2.031	-
L-Proline	0.1052	3.010	-
Glycine	0.1694	4.850	-
L-Alanine	0.1407	4.030	-
L-Cysteine	0.0628	1.800	0.80
L-Valine	0.1216	3.480	6.20
L-Methionine	0.0683	1.960	2.50
L-Isoleucine	0.1325	3.800	5.80
L-Leucine	0.5259	15.070	9.50
L-Tyrosine	0.0451	1.290	4.80
L-Phenyl alanine	0.1407	4.030	4.70
L-Tryphtophan	[c]ND	-	1.30

[a] = results are average of two amino acid auto analyzer determinations, [b] = adapted from Heine *et al.* (1991), [c] = not determined

Table 3. Sensory characteristics of *G. kola* crude and fractionated extracts

Extract	Sensory characteristics	
	Colour	Taste
ETH	dark brown	bitter
DDE	dark brown	bitter
F_1	golden yellow	tasteless
F_2	reddish yellow	slightly bitter
F_3	brown	bitter
F_4	light yellow	tasty (sweet)
F_5	sun-flower yellow	slightly bitter
F_6	light yellow	bitter

coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus faecalis and Candida albicans. This result is in agreement with earlier reports of Irvine (1965) and Sodipo *et al.* (1991), on the pharmacological and antimicrobial properties of the seeds of *G. kola*. The result of the zones of inhibition of the crude and selected fractionated extracts is shown in Table 4.

Zone of inhibition of crude ethanolic extract appeared to be higher than that of crude diethylether extract for all microorganisms with the exception of *Klebsiella pneumoniae* where the organism was more susceptible to diethylether extract. Similar results were obtained for selected fractionated (F_4, F_5) extracts. From the results (Table 4), *E.* coli was the most susceptible organism, while *S. aureus* and *C. albicans* were the most resistant to inhibition at the test concentration. This antimicrobial screening can be harnessed for the preservation of foods because some of the pathogens vulnerable to inhibition by the crude and selected fractionated extracts of the *G. kola* seeds in this study (Table 4) were associated with food deterioration and intoxication (Montefiore *et al.*, 1984).

Broth cultures were also examined for visible turbidity, the highest dilution showing no visible turbidity, was taken as the minimum inhibitory concentration (MIC) (Fig. 1). The MICs of the test organisms were 0.063-2 μg/ml of *G. kola* seeds extracts. *E. coli* was the most susceptible to inhibition (0.063 μg/ml). *S. aureus* and *C. albicans* had the highest inhibitory concentration of 2 μg/ml and 1 μg/ml, respectively.

While the minimum concentration of 0.25 μg/ml of DDE was obtained for affecting inhibition of *Bacillus cereus*, the minimum inhibition concentrations (0.5 μg/ml) of ETH and fractioned extract (F_5) on the organism were similar but less than 1 μg/ml concentration observed for inhibition of the

Table 4. Zone of inhibition of crude and fractionated extracts of the seeds of *G. kola* at recommended concentration of 0.02%

Test organisms	Zone of inhibition (mm)			
	ETH	DDE	F_4	F_5
BC	*15	12	11	11
EC	23	22	20	18
SA	R	R	R	R
KP	11	13	10	12
SF	13	10	10	11
CA	R	R	R	R

BC = *Bacillus cereus*; EC = *Escherichia coli*; SA = *Staphylococcus aureus*; KP = *Klebsiella pneumoniae*; SF = *Streptoccus faecalis;* CA = *Candida albicans*; R = resistant; * = the figures are the average of the diameters of inhibition (mm) determined

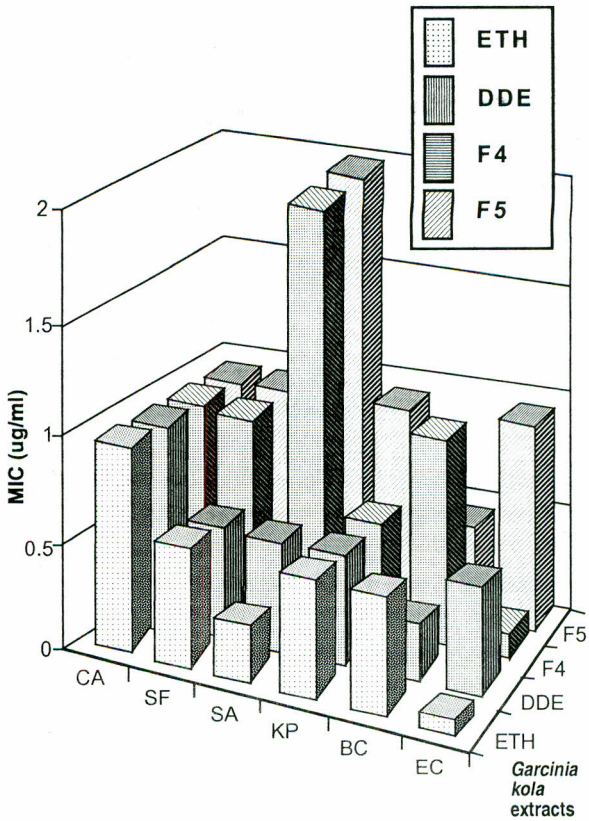

Fig. 1. Minimum inhibitory concentrations (MICs) of crude and some fractionated extracts of the seed of *G. kola* on some food borne microorganisms.

organism by fractionated extracts (F_4). The order of inhibitory concentration was: DDE < ETH = F_5< F_4. The trend of this result may be due to traces of other bioactive components that were present in DDE and F_5 that enhanced their antimicrobial activity than ETH and F_4, respectively on *B. cereus*. However, a cursory look at minimum inhibitory concentration of the additives on *E. coli* did not follow the same trend. This suggest that microbial inhibition may not depend on the number of active components in both the crude extracts or fractionated extracts only, but the chemical nature of the active components present as well as the type of microorganism have been examined. The order of susceptibility of the organism to inhibition as influenced by concentration was ETH < F_4<DDE<F_5.

The inhibitory concentration (2 μg/ml) of fractionated extracts on *S. aureus* were similar but higher than the minimum concentration (0.25-0.5 μg/ml) that inhibited the organism by the crude extracts (ETH and DDE). The order of inhibitory concentration were F_4 = F_5 > DDE > ETH.

A minimum inhibitory concentration of 0.5 μg/ml of the crude extracts and F_4 inhibited *K. pneumoniae*, while higher con-

centration (1 μg/ml) of F_5 was required to affect the inhibition. Thus inhibitory concentration was in the order:

$$ETH = DDE = F_4 < F_5$$

The crude extracts (ETH and DDE) showed a higher (0.5 μg/ml) inhibition potency against *S. faecalis* compared to a lower inhibitory activities exhibited by fractionated extracts (F_4 and F_5) (1 μg/ml) on the organism. The order of concentration of inhibition can be represented as:

$$ETH = DDE < F_4 = F_5$$

The fungus *C. albicans* required the highest (1 μg/ml) amount of crude extracts and fractionated extracts for inhibition. This result is not unexpected because yeast is more resistant to inhibition than bacteria.

Conclusion

The seeds of *G. kola* contain varying amounts of essential amino acids and elements that are important in nutrition with low amounts of antinutritional elements. Fractionation of dark brown, bitter viscous ethanolic crude extract of the seed yielded yellow non-bitter extracts with antimicrobial activities. The fractionated extracts with characteristics compatible with aesthetic appeal of food can be used to prolong storability of food from microbial deterioration.

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