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Preliminary phytochemical screening and gas chromatographic FID evaluation of *Garcinia kola* seed extracts

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ABSTRACT

The present study examined preliminary bioactive constituents and gas chromatographic FID evaluation of *Garcinia kola* seeds. Results obtained from the preliminary screening showed that the following phytochemical compounds were present flavonoids, cardiac glycosides, saponins, steroids and reducing sugars. These phytochemicals are known to play important roles in the bioactivity of medicinal plants. Results of the gas chromatographic FID evaluation showed that *Garcinia kola* contained various amount of phytochemicals. For steroids, sitosterol, campesterol, and Avenasterol had the highest concentration of 1.43 mg/100 g, 0.159 mg/100 g, 0.076 mg/100 g while in saponin, Neochlorogenin, yamogenin, tigogenin had the highest concentration of 6.286 mg/100 g, 0.910 mg/100 g, 0.017 mg/100 g. In Alkaloid lupanine, undulatine, powelline, vocangine had the highest concentration of 192.534 mg/100 g, 166.812 mg/100 g, 124.452 mg, 100 g while Dihydro-oxo-Dimethoxytyramine had the lowest concentration of 2.865 mg/100 g. Linalool, Nerol and Alpha Bergamotene had the highest concentration of 35.213 mg/100 g, 32.154 mg/100 g, 11.787 mg/100 g in Terpenes while terpineol had the lowest concentration of 0.0136 mg/100 g. Salicin and Arbutin has the highest concentration of 83.648 mg/100 g, 2.724 mg/100 g while Digitoxin has the lowest level of 0.0444 mg/100 g in glycosides. Diallythiosulphinate had the highest value of 0.0032 mg/100 g in Allicin while Apigenin was highest 42.439 mg/100 g in Lignan. Beta-crytoxanthin and Xanthophyll had the highest concentration of 436.126 mg/100 g and 314.343 mg/100 g in carotenoid. Findings showed *Garcinia kola* can be useful in the pharmaceutical and medicinal sciences.

Keywords: Phytochemicals, gas chromatographic FID, *Garcinia*, bioactive.

1. Introduction

The use of plant in traditional medicine remains the main stay of primary health care in most developing countries of the world as these plants serve as raw materials for more potent compounds of natural origin. There has however been an exponential growth in the field of herbal medicine. *Garcinia kola* seed belongs to the *Clusiaceae* family. The seed of *Garcinia kola* is chewed as masticatory, stimulant and for its bitter taste in traditional and hospitality, cultural and social ceremonies [1]. *Garcinia kola* plant is referred to as a wonder plant as every part of it has been found to be of medicinal importance. Bitter *kola* is a highly valued ingredient in African ethnomedicine because of its varied and numerous uses which are social and medicinal thus making the plant an essential ingredient in folk medicine. It has a bitter taste followed by slight sweetness. Despite its bitter taste, *Garcinia kola* nuts are commonly eaten as a snack and used for their stimulant effects, due to high caffeine content. Bitter *kola* has been used as an antidote for cases of poison or suspected poisons as it is chewed to prevent the development of any infection or poison when food is suspected to be contaminated by bacteria. The medicinal relevance of *Garcinia kola* cannot be over emphasized as several researches has been reported on its medicinal uses. Farombi *et al.*, [2] reported antiviral, antihepatotoxic and antidiabetic properties of *Garcinia kola*. Nwokocha *et al.*, [3] reported antihepatoxic effect of *Garcinia kola* supplemented diet on mercury toxicity. Ajebeson and Aine, [4] reported that *Garcinia kola* seed has been found useful in the treatment of stomach ache and gastritis. Adesuyi *et al.*, [5] reported that the medicinal properties of *Garcinia kola* seed can be classified under purgative antiparasitic and antimicrobial. Adesuyi *et al.*, [5] also reported that the

antimicrobial nature of *Garcinia kola* seed has been attributed to the Benzophenone and flavonones present in the plant while the anti-inflammatory effect is believed to result from the inhibition of the cyclooxygenase enzyme. The medicinal relevance of *Garcinia kola* seed is based mainly on the phytochemical components of the plant which is known to contain several phytochemicals noted for their medicinal importance. These phytochemicals are natural bioactive compounds found in plants. They are non-nutritive plant chemicals that have been isolated from bitter kola seed such as oleoresin, tannin, saponins, alkaloids and cardiac glycoside, bioflavonoid such as kolaflavonone and 2-hydroxyflavonoids, chromanols, [6]. Eisner, [7] reported that medicinal plants such as *Garcinia kola* are believed to be an important source of new chemical substances with potential therapeutic benefits. Keeping in view, the medicinal importance of *Garcinia kola*, and considering the fact that recent trends in controlling and treating diseases tend to favour natural medicine rather than synthetic treatment, there is need therefore, to evaluate the bioactive constituents of *Garcinia kola* seed using Gas Chromatographic Technique.

2. Materials and methods

Sample procurement and preparation

Garcinia kola seeds were sourced from a local market in Isuikwuato Local Government Area of Abia State. The samples were oven dried at 60 ^{O}C and milled to fine particles. Samples were stored in a clean air tight container for further analysis.

2.1 Gas Chromatographic Determination of Phytochemicals

2.2 Determination of Carotenoids

The carotenoid extraction were carried out following the modified method Takagi [8]. The extracts were combined and evaporated under reduced pressure and the residue were re-extracted by a mixture of diethyl ether and petroleum ether in equal ratio.

2.3 Determination of Saponins

Extraction was carried out by the method of AOAC [9]

The combined extract were concentrated to syrup under reduced pressure and then suspended in air. The suspension was extracted with petroleum ether, chloroform and 1-butanol saturated with water, successively to give the respective extract after removal of the solvent.

2.4 Determination of Sterols

Sterols analysis was carried out following the modified AOAC [9]

The sample was saponified at 95 ^{0}C for 30 minutes by using 3ml of 10% KOH in ethanol to which 0.20 ml of benzel has been added to ensure miscibility. Deionized water (3 ml) was added 2 mls of hexane was used in extracting non saponifiable particles (sterols).

2.5 Determination Phenolic Compounds.

Phenolic compounds were extracted from pulverized sample according to the method described by Ndoumou *et al*., [10]

2.6 Determination of Flavonoids

The crude methanolic extract was obtained by pouring 100 ml of the boiling methanol and (70:30) unto 10 g of the plant seed material.

2.7 Determination of Allicin

Allicin extraction was carried out using the modified method of Chehregani *et al*., [11]

2.8 Determination of Alkaloids

Alkaloids determination was carried out using the modified method of Ngounou *et al.*, [12]

After the filtration and evaporation at reduced pressure, the resultant crude extract was treated with 5% aqueous HCL of about 7.5 ml.

GC conditions for Alkaloid

2.9 Determination of Terpenes

The sample was pulverized and the terpenes constituents extracted with redistilled chloroform. The terpene were removed with 10ml of the solvent for 15 minutes. The mixture was filtered and concentrated to 1ml in the vial for Gas chromatography analysis and 1 µl was injected into the injection pot of GC.

2.10 Determination of Lignin

The lignin was removed by suction filtration and the filtrate shaking over night with hexane dichloromethane.

2.11 Determination of hydroxamic acid

The sample was pulverized and the hydroxycinnamic acid constituents extracted with methanol. The hydroxycinnamic acid were removed with 10 ml of the solvent for 15 minutes. The mixture was filtered and concentrated to 1 ml in the vial for Gas chromatographic analysis and 1ml was injected into the injection port for gas chromatography.

3. Results

3.1 Preliminary phytochemical compounds present in *Garcinia kola* crude extract.

Alkaloids	Negative
Steroids	Positive
Cardiac glycosides	Positive
Flavonoids	Positive
Tannins	Positive
Saponins	Positive
Reducing sugar	Positive

Table 1: Gas chromatographic FID Results for Carotenoids

Name	Retention time (min)	Amount (mg/100g)
Malvidin	19.516	0.143
Carotene	20.532	161.347
Lycopene	21.435	0.0508
Beta-crytoxanthin	22.356	436.126
Lutein	22.597	34.439
Anther-xanthin	24.876	0.129
Asta-xanthin	25.609	1.425
Viola-xanthin	26.351	29.672
Neo-xanthin	26.999	46.847
Xanthophylls	24.030	314.343

Table 2: Gas chromatographic FID Results for Saponin

Name	Retention time (min)	Amount (mg/100 g)
Hispogenin	17.371	0.00129
Solagenin	18.662	0.0227
Diosgenin	19.114	0.0414
Tigogenin	19.801	0.0119
Neochlorogenin	20.541	6.286
Hecogenin	21.831	0.0176
Sapogenin	22.612	34.258
Tribuloin	23.125	1.236
Yanogenin	23.975	0.910
Conyzorgin	24.797	0.001
Saponine	26.355	35.666

Table 3: Gas Chromatographic FID Results for Phenolic Compounds.

Name	Retention time (min)	Amount (mg/100g)
4-hydrobenzenaldehyde	8.899	6.019
4-hydrobenzoic acid	12.360	26.763
4-hydroxylbenzoic acid methyl ester	13.541	13.926
Vanillic acid	15.153	53.652
Gallic acid	16.242	33.104
Ferulic acid	18.048	94.553
Capsaicin	19.675	0.0152
Rosmarinic acid	20.592	16.787
Tannic acid	22.597	48.513

Table 4: Gas Chromatographic Results of Flavonoids

Name	Retention time (min)	Amount (mg/100g)
Catechin	13.736	1.159 mg/100g
Resveratrol	15.056	0.0045 1 mg/100 g
Genistein	15.499	4.990 mg/100 g
Apigenin	16.140	0.0010 mg/100 g
Butein	16.671	0.00000907
Naringenin	16.967	0.00000900
Biochanin	17.087	0.0000111 mg/100 g
Luterlin	17.763	0.00578 mg/100 g
Kaemterol	18.046	26.099 mg/100 g
(-) Epicatechin	19.511	2.599
(-) Epigallocatechin	20.618	3.074
Quercetin	21.813	38.902
Ellagic acid	24.434	0.432
Nobiletin	25.460	0.00000457
Tangertin	26.472	0.00000455
Artemetin	26.821	0.00000266
Silymarin	27.051	0.00000708
Kaemterol-3-arabinoside	27.275	0.00240 mg/100 g
Quercitrin	27.423	5.700 mg/100 g
Rutin	28.092	17.484 mg/100 g
Hesperidin	28.317	0.00000134

Table 5: Gas Chromatographic FID Results for Allicin

Name	Retention Time (min)	Amount (mg/100 g)
Diallythiosulphinate	16.098	0.00328
Methylallythiosulphinate	16.926	0.00158
allymethylthiosulphinate	18.535	0.00055

Table 6: Gas Chromatographic FID Results for Alkaloids

Name	Retention time (min)	Amount (mg/100 g)
Choline	7.291	0.844
Theophylline	7.901	20.186
Angustitoline	8.952	7.437
Sparteine	9.293	192.534
Lupanine	11.041	5.383
B-Alphahudrorhombifoline	11.286	8.513
9-Octadecenamide	12.936	39.337
Augustamine	14.918	38.609
Oxoassoanine	15.037	4.596
Capsaicin	16.036	49.609
Cinchonidine	16.246	7.026
Crinane-3-Alpha-ol	16.491	32.729
Buphanidrine	16.669	25.402
Indicine-n-oxede	17.547	124.452
Powelline	18.590	166.817
Undulatine	19.678	16.331
Ambelline	20.473	20.181
6-hydroxypowelline	21.130	5.529
Nctidine	21.328	5.859
Crinamidine	21.821	3.332
Ibeta+2-Beta, Epoxyambelline	22.366	3.883
6-hydroxybuphanidrine	23.968	26.472
Epoxy-3, 1-dimethoxycrinane-11-one	26.837	13.567
Akuammidine	26.952	10.190
Echitammidine	24.062	106.383
Voacangine	27.649	2.938
Nutraphyline	28.527	0.1432

Table 7: Gas Chromatographic FID Results for Terpenes

Name	Retention time (min)	Amount (mg/100 g)
Alpha pinene	9.871	0.0313
Beta pinene	11.021	0.098
Cis Ocimene	11.454	0.0229
1,8-cineol	15.617	4.101
Borneol	15.617	0.167
Linalool	15.888	35.213
Nerol (gerancol)	16.041	32.154
Alpha Terpineol	16.304	0.0136
Ethyl cinnamate	16.016	2.072
Borneol Acetate	17.614	0.376
Geranyl Acetate	17.868	0.0150
Taraveron	18.845	0.031
Alpha Amyrin	19.684	7.694
Beta Amyrin	20.197	6.177
Alpha Bergamotene	20.638	11.787

Table 8: Gas Chromatographic FID Results for Lignan

Name	Retention Time (min)	Amount (mg/100 g)
2-ally1-5ethoxy-4-methoxylphenol	11.746	0.00719
(9E, 12E, 15E) -9, 12, 15-Octadecatrien-1-01	14.218	0.00501
Apigenin -4, 7-methyl ether	16.110	42.439
Dehydroabietic acid	17.992	0.0245
Retusin	19.056	2.498
Galgravin	20.417	2.423
Epieudesmin	22.310	0.141
Sakuranin	23.999	0.0932

Table 9: Gas Chromatographic FID Results for Hydroxycinnamic compounds

Name	Retention Time (min)	Amount (mg/100 g)
P. coumarin	8.211_{WT}	3.134 mg/100 g
P. coumaric acid	11.433_{WT}	44.439 mg/100
Caffeic acid	14.394_{WT}	25.291 mg/100 g
Scopoletin	15.786_{WT}	4.870 mg/100 g
Chlorogenic acid	18.246_{WT}	15.511 mg/100 g
Chicoric acid	19.663_{WT}	4.987 mg/100 g

4. Discussions:

Results obtained from the preliminary phytochemical screening showed presence of steroids, flavonoids, cardiac glycosides, tannins, saponins. Steroidal compounds have been reported by [13] to be associated with such compounds as sex hormones. Tannins has been observed to have remarkable activity in cancer prevention and anticancer [14]. In addition to this [15] showed tannins to be useful in treatment of inflamed or ulcerated tissues. Saponin was also present in *G. kola* extract and has supported the usefulness of this plant in managing inflammation. *G. kola* ranked well among the medicinal plants used routinely among many tribes in Nigeria and some part of Africa for the treatment of infectious diseases. Flavonoids has been shown to exhibit anti-inflammatory, antiangionic, anti-allergic effects, analgesic and antioxidant properties. The gas chromatographic FID analysis of *Garcinia kola* seeds revealed that *Garcinia kola* contain several phytochemicals. These bioactive compounds in *Garcinia kola* such as terpenes has been shown to be useful in treatment of inflamed or ulcerated tissues [16]. Alkaloids has been shown to act as pain relievers, and contain antimicrobial properties. *Garcinia kola* is often used to treat symptoms of colds,

4.1 Conclusion

Findings of these research showed that *Garcinia kola* contain varying amount of phytochemicals and can be useful in pharmaceutical and medicinal sciences, in drug manufacturing and in various manufacturing industries as raw materials. These bioactive phytochemicals contained in *Garcinia kola* seeds are the critical components of the medicinal relevance of these seeds.

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