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Comparative Study of the Chemical Constituents of Bitter Kola (*Garcinia Kola*) and Cola Nut (*Cola Acuminata*) Seeds Extracts

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Abstract

Powdered form of the dried seeds of *Garcinia kola* and *Cola acuminata* were separately cold extracted with dichloromethane (DCM). Phytochemical screening of the DCM extracts of the seeds showed the presence of alkaloids, saponins, flavonoids, tannins, steroids and glycosides. The study also revealed the presence of terpenoids in *G. kola* which was apparently absent in *C. acuminata.* The chemical constituents were investigated using Thin Layer Chromatography (TLC) while Isolation and purification of the major constituents of the two seeds were carried out using Column Chromatography and Preparative Thin Layer Chromatography. The FT-IR analyses revealed O-H stretching vibration of an alcohol at 3427 cm^{-1}, C=O stretching of an ester at 1703 cm^{-1}, N-H stretch of amide at 3111 cm^{-1} in *G. kola* isolate and the O-H stretching band of an alcohol at 3346 cm^{-1}, the C=C stretching of an alkene at 1685 cm^{1} and aliphatic C-H stretching at 2924 cm^{-1} in *C. acuminata.* The compounds identified from *G. kola* through the NIST data are as follows: caffeine (RT=13.000, 31.41%), 9-octadecenoic acid, (E)- (RT=14.204, 4.42%), 9-octadecenamide, (Z)- (RT=16.120, 8.89%), Phthalic acid, di (2-propylpentyl) ester (RT=16.919, 6.37%), Beta-amyrin (olean-12-en-3-ol) (RT=17.525, 24.53%) and Alpha-amyrin (RT=18.744, 21.97%) while *C. acuminata* identified n-hexadecanoic acid (RT=12.871, 32.02%), 9- octadecenoic acid, (E) - (RT=14.190, 28.94%), 9-octadecenamide (Z) - (RT=16.427, 1.65%), and phthalic acid, di (2-propylpentyl) ester (RT=16.918, 19.09%).

Keywords: Extraction, kola, isolation, purification, chromatography.

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1. INTRODUCTION

Nuts are strong radical scavengers and can be eaten as part of a diet to alleviate the symptoms of chronic and degenerative diseases reported to be on the increase in developing countries (Ogunlade et al., 2014). Bitter kola *(Garcinia kola*) is a member of the family *Guittiferae* known as '*Namijin Goro* or *Cida Goro*' in Hausa (Northern part of Nigerian) and '*Orogbo*' in Yoruba (Western part of Nigeria), '*Adili, Aku ilu* or *Ugugolu*' in Igbo (Iwu et al*.,* 1999). The seeds have a bitter taste; hence, it is called bitter kola (common name) in Nigeria (Nebula and Mbakwe, 2001). The seeds are edible, relieve cough and hoarseness of the vocal cords and have been consumed as a stimulant (Tita et al., 2001; Mackeen et al., 2002; Okwu and Ekeke, 2005).

Cola nut belongs to plant family of *Sterculiaceae* and over 125 species of cola nut have been identified in which out of these species only two are well known for their commercial purposes and ceremonial roles in the society, namely: *Cola acuminata* (white and red varieties) known as obi *Abata, Cola nitida* known as obi *Gbanja* in Yoruba (Kouame and Sacande, 2006; Asogwa et al, 2012). *Cola* has common names such as cola nut in English, obi-*gbanja*/ *abata* in Yoruba, Colatier in French, *goro* in Hausa (Kouame and Sacande, 2006; Asogwa et al*.*, 2012).

The main flavonoids content isolated from *G. kola* seed is kolaviron, a defatted ethanol extract from the seeds. Kolaviron is a mixture which contains *garcinia* biflavonoids and kolaflavanone (Nwakwo et al., 2000; Iwu et al., 1999). It has been reported by Adaramoye et al., (2005); Farombi et al., (2005); Olaleye et al., (2000) that the flavonoids and phenolic compounds present in the plant are responsible for antioxidants, anti-inflammatory, anti-tumor, anti-hepatotoxic, anti-ulcer and anti-microbial properties exhibited by the plant. Administration of GK seed extract caused an increase in testosterone production in Sprague–Dawley rats which is thought to be due to its antioxidant properties (Akpantah et al., 2005). Traditionally, it is said that regular consumption of *Garcinia kola* nut lowers blood glucose levels and improves the complications of diabetes mellitus. The

seed is chewed whole and one to three whole seeds may be taken a day (Adaramoye et al., 2005).

The husk and testa of the Kola nut species has antioxidant properties and thus can exert several beneficial effects by virtue of these properties. Regular consumption of foods and beverages exhibiting antioxidant activity can help reduce the harmful effects of free radicals and oxidative stress (Fabunmi and Arotupin, 2015). *Cola acuminata* has higher antioxidant activity and total phenolic content (Atolaye et al., 2009). Cola nut seeds have been used to control vomiting in pregnant women, and also used by drivers, farmers, students and people as a principal stimulant to keep awake and withstand fatigue that engaged in fatigue works (Chukwu et al., 2006). According to Muhammad and Fatima, (2014), aqueous and methanol extracts of red and white variety of cola nut showed antibacterial activity against *Streptococcus anginosus,* gram positive bacteria which is a member of the viridian Streptococcus (Ryan and Ray, 2004).

However, no report has been sighted on the comparative study of the chemical constituents of *Garcinia kola* and *Cola acuminata* alongside each other. This work aims at carrying out cold extraction of *Garcinia kola* and *Cola acuminata* seeds separately, identify, isolate the main components in *Garcinia kola* and *Cola acuminata* seeds respectively, characterize the isolates using various spectroscopic techniques and compare the characterized chemical constituents of *Garcinia kola* and *Cola acuminata* seeds.

2. MATERIALS AND METHOD

Materials and Reagents

The major raw material used in this work includes fresh bitter kola and cola nut seeds obtained from Oja-Oba in Ilorin, Kwara State, Nigeria. The seeds were authenticated in the Plant Biology Department, University of Ilorin, Ilorin, Nigeria. All reagents used were of analytical grade.

2.1 Extraction procedure

The mesocarps of the seeds were peeled, washed with water, air dried at room temperature and ground to powder with mortar and pestle to increase the surface area for maximum extraction. Two hundred grams (200 g) each of powdered *Garcinia kola* and *Cola acuminata* seeds was weighed into separate extraction bottles and 500 mL of dichloromethane was added to the powdered bitter kola and cola nut seeds respectively.

2.2 Phytochemical Analysis

Qualitative phytochemical screening was carried to determine the presence of alkaloids, saponins, glycosides, tannins, flavonoids, steroids and terpenoids using the standard methods described by Trease and Evans, (1997).

2.3 Chromatographic analyses

The thin layer chromatography of the extracts (*G. kola* and *C. acuminata*) was carried out to identify various chemical constituents present. The chromatograms were sprayed with vanillin reagent followed by heating where required to check colour reaction. Column chromatography was carried out for purification and isolation of desired compounds from the crude mixture. Silica gel slurry was prepared and used to coat the PTLC plates manually. This was done by mixing adequate amount of silica gel with binder (calcium sulphate) in appropriate quantity of warm water, shaken thoroughly to form slurry. The slurry was then applied on pre-washed, cleaned and dry the preparative thin layer glass plates (24 cm x 24 cm), allowed to air dry for 24 hours and activated in a regulated oven for 30 minutes at 120^{o}C

2.5 Recrystallization of *G. kola* precipitate

The concentrated filtrate of the DCM crude extract of *G. kola* was left standing for three days and crystals precipitated out. The crystals were purified by recrystallizing with DCM until pure crystals were obtained. The melting point of the crystals was determined.

2.6 Structural elucidation

The isolates were characterized using data obtained from Fourier-Transform Infrared (FTIR) Spectroscopy using Shimadzu 8400s (Schimadzu Corporation, Kyoto Japan, Gas Chromatography coupled with Mass Spectrometry (GC/MS) and X-Ray Diffraction.

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis

The qualitative phytochemical analyses results of the DCM extracts of *G. kola* and *C. acuminata* respectively presented in table 1 revealed the presence of tannins, glycoside, flavonoids, steroids, saponins. This is in agreement with the findings of Adegboye et al., (2008); Adesuyi et al., (2012); Olaleye et al., (2000) and Ogunmoyole et al., (2012). The presence of saponin in *G. kola* extract supports the usefulness of *G. kola* and *C. acuminata* in managing inflammation. The presence of steroids *G. kola* is of importance and interest due to their relationship with such compounds as sex hormone (Okwu, 2001). The astringent nature of *G. kola* has been ascribed to the presence of tannin in treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003; Chikezie et al., 2008). Also, Tannins has been observed to have remarkable activity in cancer prevention, treatment of inflamed tissues, treatment of ailments caused by microorganisms (Li et al., 2003). The presence of glycoside in *G. kola* and *C. acuminata* has been attributed to its ability to treat cough, chest pain and other cardiac infections (Yukari et al., 1995). From table 1, phytochemical analyses revealed that terpenoids are not present in *Cola acuminata*. This is in agreement with investigation carried out by Dewole et al., (2013)

Table 1: Qualitative phytochemical screening of *G. kola* and *C. acuminata*

	G. kola	***C. acuminata***
Tannin	+	+
Saponin	+	+
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+
Terpenoids	+	–
Steroids	+	+

Keys:

(+): metabolite present

(-): metabolite absence

3.2 Infrared Spectra of Isolated Compounds

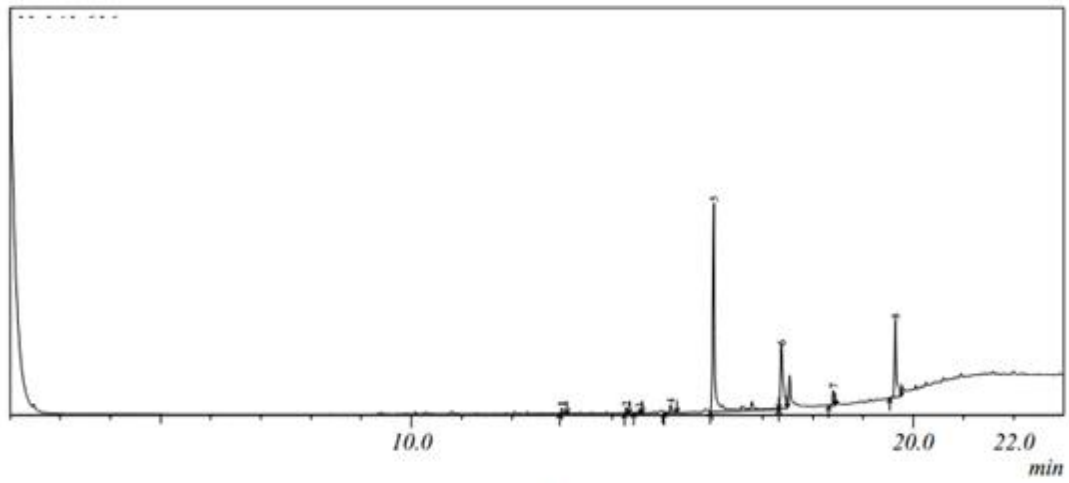
The IR spectrum of the BK-C is interpreted thus: the O-H stretching vibration indicated at 3427 cm^{-1} can be attributed to the presence of an alcohol, the C=O stretching at 1703 cm^{-1} can be attributed to that of an ester. The C=C absorption band at 1548 cm^{-1} is alluded to that of non-conjugated alkene while the vibration stretching at 1654 cm^{-1} can be attributed to the presence of conjugated alkene. The N-H stretch at 3111 cm^{-1} indicates the presence of amide while the C-H bend showed at 1483 cm^{-1}
The O-H stretching band at 3346 cm^{-1} is that of an alcohol. The C=C stretching at 1685 cm^{-1} indicated the presence of an alkene while the C=C stretching at 1602 cm^{-1} can be attributed to an aromatic. The aliphatic C-H stretching showed at 2924 cm^{-1}

3.3 GC-MS Analyses Results

GC-MS analyses of the *G. kola* crystals were carried out and three main peaks were highlighted. The seven major peaks correspond to seven different compounds. The peaks according to NIST library matching are caffeine (RT=13.000, 31.41%), 9-octadecenoic acid, (E)- (RT=14.204, 4.42%), octadecanoic acid (RT=14.343, 2.30%), 9-octadecenamide, (Z)- (RT=16.120, 8.89%), 9-octadecenamide, (Z)- (RT=16.120, 8.89%), Phthalic acid, di (2-propylpentyl) ester (RT=16.919, 6.37%), Beta-amyrin (olean-12-en-3-ol) (RT=17.525, 24.53%), Alpha-amyrin (RT=18.744, 21.97%) all constituting 87.65% of the total.
A unique constituent of the isolate is the presence of caffeine, being a major constituent of the *G. kola*. Though caffeine has not been properly reported to be present in bitter kola unlike cola nut and some other varieties of cola, the confirmation of the presence of caffeine in this report is in agreement with the findings of Onochie and Stanfield, (1960); Odebunmi et al, (2009) which is believed to be an aphrodisiac. Another unique compound in the *G. kola* is 9-octadecenamide, (Z) which to the best of our knowledge has not been reported to be found in bitter kola (*G. kola*). Also, amyrin (beta-amyrin and alpha amyrin esters) constituting a total of 46.5% are other unique compounds identified for bitter kola in this report and is noteworthy because they have not been reported isolated from any part of *G. kola* as at the time of this report.

Six components were identified from *C. Acuminate* in the figure 2 presented below. The compounds identified through the NIST data are as follow: n-hexadecanoic acid (RT=12.871, 32.02%), 9-octadecenoic acid, (E) - (RT=14.190, 28.94%), octadecanoic acid (RT=14.334, 12.96%), 9-octadecenamide (Z) - (RT=16.427, 1.65%), hexadecanoic acid (RT=16.699, 5.32%), phthalic acid, di (2-propylpentyl) ester (RT=16.918, 19.09%). 9-octadecenamide, (Z) being a unique compound identified by the GC-MS analysis of *G. kola* and *C. acuminata* seeds is known for its anti-inflammatory activity and antibacterial activity, this in agreement with Hodek et al., (2002), this is in agreement with the FT-IR results which revealed the functional group of amide and also, the phytochemical test results revealed the presence of flavonoids in *G. kola* and *C. acuminata* seeds.

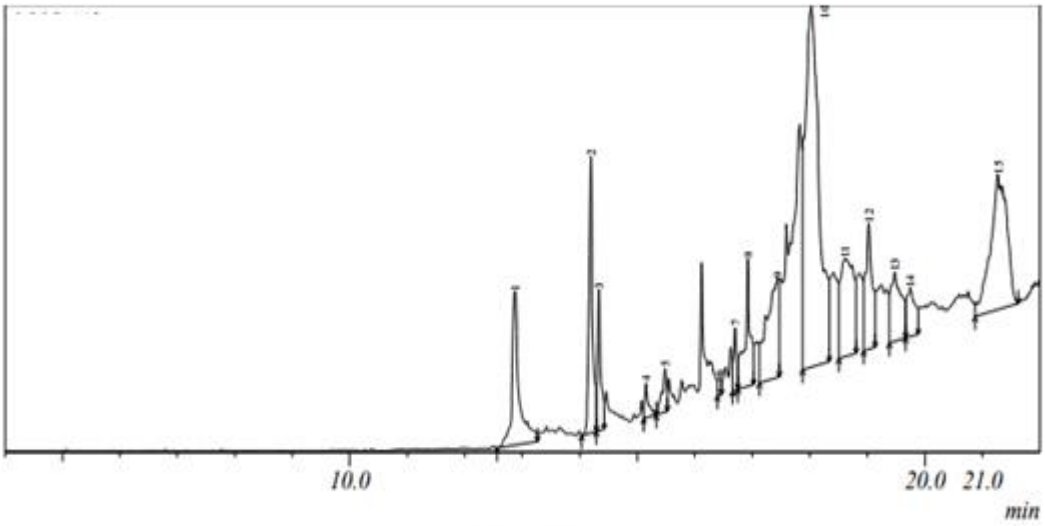
One of the unesterified fatty acids identified in *G. kola* crystal is hexadecanoic acid. From figure 2, the molecular weight m/z 256 of the compound represents the extrusion of carboxylic group. Peaks at m/z 239, 227, 199, 185 to 73 represent the breaking array of 14 atomic number unit (amu) of CH_2 groups. However, the most important peak in the mass spectrum of an aliphatic acid is the peak at m/z =60, which results from a γ-hydrogen shift (γ-H). The confirmation of aforementioned compounds by GC-MS analysis of *Cola acuminata* is in agreement with the GC-MS analysis carried out on the ethanolic crude extract of cola nut (*Cola nitida*) by Salahdeen et al., (2015)



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	12.997	12.958	13.100	354482	1.04	145446	1.37	2.44		Ibuprofen
2	14.282	14.242	14.342	370761	1.09	164835	1.56	2.25	V	Tetradecanoic acid
3	14.543	14.425	14.600	194877	0.57	112952	1.07	1.73	V	cisZ-11,12-Epoxytetradecan-1-ol
4	15.165	15.033	15.292	657307	1.94	217025	2.05	3.03	V	Pentadecanoic acid
5	16.025	15.958	17.317	18886810	55.62	5712599	53.93	2.96	V	n-Hexadecanoic acid
6	17.373	17.317	17.492	6598378	19.43	1707626	16.12	3.86		9,12-Octadecadienoic acid (Z,Z)-
7	18.408	18.308	18.450	961768	2.83	407341	3.85	2.36		9-Octadecenamide, (Z)-
8	19.644	19.533	19.758	5929941	17.46	2125308	20.06	2.79	V	9-Octadecenamide, (Z)-
				33954324	100.00	10593132	100.00			

Figure 1. GCMS spectrum of C. Acuminate isolate



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	12.871	12.558	13.258	20675034	7.16	2383001	8.64	8.68	V	n-Hexadecanoic acid
2	14.190	14.025	14.283	18686429	6.47	4267507	15.47	4.38		9-Octadecenoic acid, (E)-
3	14.334	14.283	14.425	8375214	2.90	2180651	7.91	3.84	V	Octadecanoic acid
4	15.146	15.108	15.325	2363539	0.82	524302	1.90	4.51	V	Hexadecanoic acid, 2-propyl-, methyl ester
5	15.480	15.325	15.517	3505886	1.21	667229	2.42	5.25		Butyl 9,12-octadecadienoate
6	16.427	16.392	16.467	1055522	0.37	282515	1.02	3.74	V	9-Octadecenamide, (Z)-
7	16.699	16.658	16.750	3437421	1.19	968146	3.51	3.55	V	Hexadecanoic acid, 2-hydroxy-1-(hydroxym
8	16.918	16.750	17.017	12348919	4.27	1980586	7.18	6.23	V	Phthalic acid, di(2-propylpentyl) ester
9	17.445	17.125	17.467	22329301	7.73	1519796	5.51	14.69	V	9-Octadecenamide, (Z)-
10	18.025	17.867	18.333	94458450	32.70	5519915	20.01	17.11	V	3.beta.-Myristoyllolean-12-en-16.beta.-ol
11	18.608	18.500	18.800	24275963	8.40	1506857	5.46	16.11	V	A'-Neogammacer-22(29)-ene
12	19.029	18.933	19.133	15203887	5.26	1911249	6.93	7.95	V	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,3
13	19.481	19.383	19.667	13495284	4.67	1061695	3.85	12.71	V	Stigmast-5-en-3-ol, oleate
14	19.750	19.667	19.883	7431791	2.57	746587	2.71	9.95	V	Tetracosamethyl-cyclododecasiloxane
15	21.273	20.867	21.617	41264171	14.28	2064150	7.48	19.99	V	Coprostan-16,22-epoxy-3.beta.-ol
				288906811	100.00	27584186	100.00			

Figure 2. GCMS of G Kola isolate

4. CONCLUSION

The GC-MS analyses revealed the presence of caffeine in *G. kola* which is being reported for the first time in the present study. The result showed the presence of Beta-amyrin (olean-12-en-3-ol) and Alpha-amyrin in *G. kola* crystals which has not been reported as at the time of this report. β-amyrin is known for its medicinal purposes as it is used as antibiotic in medicines and major ingredient in fragrance. It is also used as anticancer, antiparasitic, antiallergenic, antispasmodic, antihyperglycemic and as herbicide, fungicide. The presence of various bioactive compounds justifies the use of the *G. kola* and *C. acuminata* seeds for combatting various ailments by traditional practitioners. n-hexadecanoic acid, a major compound identified in G. kola seeds is said to possess antioxidant activity. The spectroscopic analyses applied in elucidating the chemical constituents present in *G. kola* and *C. acuminata* revealed that they possess different chemical constituents with rather few similarities. The presence of secondary metabolites in the two extracts i. e flavonoids is the confirmation that the two extracts have anti-inflammatory, antiangionic, anti-allergic effects, and analgesic and antioxidant properties. The Presence of tannins in the extracts is remarkable, considering the potential for cancer prevention, anticancer, treatment of inflamed tissues and treatment of ailments caused by microorganisms. Also, the presence of glycoside of *G. kola* and *C. acuminata* will serve as basis for application in treating cough, chest pain and other cardiac infections

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