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Identification of compounds in *Garcinia kola* (Heckel) Fruit Pulp Using Gas Chromatography-Mass Spectrometry

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Garcinia kola fruit pulp is consumed by few human populations as food for its medicinal usefulness. The aim of this work was to identify compounds in dichloromethane/methanol and methanol extracts of *G. kola* fruit pulp using Gas Chromatography-Mass Spectrometry analysis. Twenty eight bioactive compounds were identified in the dichloromethane/methanol extract while thirty eight compounds were identified in the methanol extract. The most abundant compounds in the methanol extract were 3-Methyl-2,5-furandione, 5-hydroxymethyl furfural and 4,5-diethenyl-2,2-dimethyl-1,3-dioxolane which had concentrations of 17.37%, 14.53% and 10.55% respectively. The most abundant compounds in the dichloromethane/methanol extract were lupeol, n- hexadecanoic acid (i.e palmitic acid) and (Z) Octadec-9-enoic acid (i.e oleic acid) which had concentrations of 37.53%, 12.94% and 10.24% respectively. Results indicated that polarity of the solvents used in extraction influenced the relative abundance and type of compounds extracted. Various compounds identified are known to have varying bioactivities such as being anticancer, antioxidant

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and anti-sickling. Therefore the presence of these compounds in *Garcinia kola* fruit pulp could be useful in the preparation of functional foods and neutraceuticals in preventing and treating debilitating and chronic human diseases.

Keywords: Garcinia kola fruit pulp; gas chromatography-mass spectrometry; bioactive compounds; fatty acids; triterpenoids.

1. INTRODUCTION

Plants play a major role in primary health care as therapeutic remedies in developing countries [1]. They serve as source of medicine and an important component in health care system [2]. This stems from the fact that aside from providing vitamins and minerals, plants contain phytochemicals which are secondary metabolites. These phytochemicals have various bioactivities such as being antioxidants, antinutritional or cytotoxic. Antioxidants are substances used by the body to protect itself from damage caused by free radicals, whose accumulation has been linked to several diseases such as heart diseases [3], liver diseases and cancers [4]. The antinutritional activity of a bioactive compound is based on its ability to inhibit the absorption and utilization of nutrients. Currently, there is upsurge interest in the knowledge of bioactive compounds present in plants and hence their beneficial potentials as neutraceuticals and functional foods hence, having positive health promoting effects.

Garcinia kola Heckel belongs to the family formally Guttiferae now Clusiaceae [5,6]. It is called 'Akuilu' in Igbo land South-Eastern Nigeria, 'Orogbo' in Yoruba in Western Nigeria while it's English name is 'Bitter kola' [5]. The plant grows in parts of Cameroun, Nigeria, Gabon and other African countries with coastal rain forests [7]. *G. kola* tree grows up to 12 m high with fruits produced from July to October. The fruits are about 2.5 inches in diameter and have subglobose shape which may be yellow or red in color. It contains ellipsoid brown seeds embedded in an orange colored pulp [8]. The seed is a highly valued ingredient in African ethno-medicine because of its varied and numerous uses which are social and medicinal [9], while the fruit pulp is used in the treatment of jaundice and high fever in some localities [7]. The pulp has been reported to be a potential substrate for the production of ethanol [10]. It's nutrient, phytochemical and physicochemical properties has been evaluated [11]. However, there is dearth of information on type of bioactive compounds present in the fruit pulp. It is in view of this that a study was conducted to identify bioactive compounds present in two solvent extracts namely dichloromethane/methanol and methanol of *G. kola* fruit pulp.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Samples

Mature *Garcinia kola* fruit was procured in the month of November, 2016 from Uturu in Isiukwuato Local Government Area of Abia State, Nigeria. It was identified by a taxonomist in the Department of Botany, Abia State University, Uturu, Nigeria. Fruits were allowed to ripen before they were cut open and seeds were extracted while the pulp was recovered by scrapping using a clean spoon. It was oven dried at 40°C for 48 h. Subsequently, the oven dried *G. kola* fruit pulp was pulverized in a blender.

2.2 Extraction of Bioactive Compounds

It was performed according to the method described in by [12]. Methanol and dichloromethane/methanol (1:1) were used to extract bioactive compounds. 15 g the sample was put into two labeled conical flasks respectively. 300 ml of methanol and 300 ml dichloromethane/methanol were added to the conical flasks containing the samples respectively and shaked vigorously. Each flask containing the sample and solvent were covered using aluminum foil and allowed to stand for 24 h at room temperature. Each sample mixture was filtered through Whatman filter paper No 1. The respective extracts were concentrated by evaporating excess solvent. Hence, two extracts of *G. kola* fruit pulp were obtained. These were subjected to Gas Chromatography-Mass Spectrometry analysis for the separation and identification of compounds respectively.

2.3 Gas Chromatography-Mass Spectrometry Analysis

Extracts of *G. kola* fruit pulp were subjected to gas chromatography-mass spectrometry analysis

(GC-MS). This was done using GC-MS (Model QP 2010 series Shimadzu, Japan) equipped with Optima 5 ms fused capillary column of 30 mm length, 0.25 mm diameter and 0.25 µm film thickness. Helium (99.99%) was used as carrier gas. The temperature programming was set with initial column oven temperature of 60°C hold time of 2 mins by 120°C/min to a final temperature of 300°C withhold time for 2 mins. 2.0 µl of the *G. kola* fruit pulp extracts were injected using a Hamilton syringe into the GC for total ion chromatographic analysis with spilt injection technique (3:1). The injector temperature was 250°C, the ion source temperature was 200°C with an interface temperature of 280°C, and recorded over a scan range of 45 to 650 m/z with electron impact ionization energy of 70ev. Total running time of GC-MS for the methanol extract was 20min while for the dichloromethane/methanol extract was 22 mins. The relative percentage of each extract constituents were expressed as percentage with peak area normalization.

Compounds in the were identified by mass spectroscopy by comparing their retention indices and mass spectra fragmentation patterns with those stored on the computer library. National Institute of Standards Technology (NIST/EPA/NIH Mass Spectral Library, Version 2.0). Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

3. RESULTS AND DISCUSSION

GC-MS analysis revealed the presence of 28 compounds in dichloromethane/methanol and 38 compounds in methanol extracts of *Garcinia kola* fruit pulp respectively. Peak number, retention time, compound name, molecular weight and molecular formula are stated in their various tables.

Table 1 shows compounds identified in dichloromethane/methanol extract of *G. kola* fruit pulp which represented the hydrophobic (i.e lipophilic) fraction. Three compounds were identified as the major bioactive compounds. They are lupeol and it had a relative abundance of 37.53% and eluted as represented at peak 27, palmitic acid (i.e n-hexadecanoic acid) had a relative abundance of 12.94% and eluted as represented at peak 16 and oleic acid (i.e 9-octadecenoic acid-Z-) had a relative abundance of 10.42% and eluted as represented at peak 20. These represented 60.89% of all the compounds in the hydrophobic fraction. Lupeol had the highest relative abundance. It is a triterpenoid phytosterol and has been shown to have effective therapeutic potentials and has no toxicity to normal cells and tissues [13]. It has beneficial activity against inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic toxicity [14]. The presence of lupeol in *G. kola* fruit pulp entails that it's consumption will proffer health benefits aforementioned for it.

(Z) Octadec-9-enoic acid (i.e oleic acid, 10.42%) and n- hexadecanoic acid (i.e palmitic acid, 12.94%) acids were the major fatty acids identified in dichloromethane/ methanol extract of *G. kola* fruit pulp. [15] reported octadecanoic acid (i.e stearic acid, 21.72%), n- hexadecanoic acid (i.e palmitic acid, 20.31%) and (Z) Octadec-9-enoic acid (i.e oleic acid, 19.32%) were the major fatty acids found in *Garcinia tinctoria* fruit pulp. 9-Octadecenoic acid has biological activities such as being anti-inflammatory, anti-androgenic, cancer preventing, dermatigenic, hypocholesterolemic, anaemiagenic, insectifuge as well as being a 5-Alpha reductase inhibitor [16]. n- hexadecanoic acid has been reported to show selective toxicity to human leukemic cells as well as anti tumor activity and is suggested to be a lead compound in anti cancer drugs [17]. Other fatty acids and fatty acid esters identified were (E)-Methyl 9-octadecenoate (i.e eliadic acid methylester, 5.08%), octadecanoic acid (i.e stearic acid, 4.59%), pentadecanoic acid (0.39%), tetradecanoic acid (i.e myristic acid, 0.90%), cis-9-hexadecenoic acid (i.e palmitoleic acid, 1.49%), hexadecanoic acid methyl ester (2.54%) and eicosanoic acid (i.e arachidic acid, 0.46%). (E)-Methyl 9-octadecenoate is a trans isomer of (Z) Octadec-9-enoic acid. It increases plasma cholesteryl ester transfer protein activity which lowers high density lipoprotein cholesterol [18] but has a positive action of being anti carcinogenic [19]. This entails that the presence of these fatty acids in *G. kola* fruit pulp will be of benefit in human nutrition.

Table 2 shows results on compounds identified in methanol extract of *G. kola* fruit pulp. Three compounds were identified which represented the hydrophilic bioactive fraction. They are 3-Methyl-2,5-furandione (i.e. Citraconic anhydride). It had a relative abundance of 17. 37% and eluted as represented at peak 4, 5-hydroxymethyl furfural had a relative abundance of 14.53% and eluted as represented at peak 18 and 4,5-diethenyl-2,2-dimethyl-1,3-dioxolane had

a relative abundance of 10.55% and eluted as represented at peak 20. These represented 42.45% of the compounds in the hydrophilic fraction of *G. kola* fruit pulp. Similarly, [20] reported that the major bioactive compounds identified by Gas Chromatography- Mass Spectrometry analysis in methanol extract of *Garcinia dulcis* fruit were 5-hydromethyl furfural (39.61%) and 3-methyl-2,5-furandione (26.24%) together with xanthones and flavonoids. 5-Hydroxymethyl furfural is a heterocydic aromatic aldehyde which is widely present in foods [21]. It is produced by the degradation of hexoses through Millard reaction during heat treatment of foods containing reducing sugars and amino acids in an acid environment [22]. It also exists in coffee, honey, dried fruits, fruit juices and flavoring agents [21] as well as in bakery products, malt with very high concentrations in food items such as dried fruits, caramel and vinegar [23]. 5- Hydroxymethyl furfural was used as a marker of quality in processed fruits, coffee,

Table 1. Compounds identified in dichloromethane/methanol extract of *Garcina kola* fruit pulp by GC-MS

Peak no	RT (mins)	Name of compound	Molecular formula	Molecular weight	Relative abundance (%)
1	4.821	dihydro -3-methylene -2,5-Furandione	$C_5H_4O_3$	112	1.55
2	4.883	Butanedioyl dihydrazide	$C_4H_{10}N_4O_2$	146	0.49
3	6.119	(-)-Menthoxyacetyl chloride	$C_{12}H_{21}ClO_2$	232	0.64
4	6.700	trans -5-methyl-2-(1-methyethyl)-Cyclohexanone	$C_{10}H_{18}O$	154	0.32
5	6.940	4-Methyl itaconate	$C_6H_8O_4$	144	1.95
6	7.967	5-Hydroxymethyl furfural	$C_6H_6O_3$	126	0.69
7	9.857	4,5-diethenyl-2,2-dimethyl-1,3-dioxolane	$C_9H_{14}O_2$	154	2.68
8	10.600	2-Pyridineacetic acid, hexahydro	$C_7H_{13}NO_2$	143	0.36
9	10.785	Timethyl citrate	$C_9H_{14}O_7$	234	0.89
10	11.507	1-Methyl-1-(2-tridecyl)oxy-1-silacyclopentane	$C_{18}H_{38}OSi$	298	0.54
11	12.289	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.90
12	12.936	2,2-dimethyl-5-(3-methyloxiran-2-yl)cyclohexa-1-one	$C_{11}H_{18}O_2$	182	0.36
13	12.994	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.39
14	13.382	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	2.54
15	13.561	cis-9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	1.49
16	13.692	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.94
17	14.300	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.46
18	14.888	(E)-Methyl 9-octadecenoate	$C_{19}H_{36}O_2$	296	5.08
19	14.622	Methyl 10-methylheptadecanoate	$C_{19}H_{38}O_2$	298	0.32
20	14.800	(Z) Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	10.42
21	14.909	Octadecanoic acid	$C_{18}H_{36}O_2$	284	4.59
22	15.932	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258	0.86
23	16.867	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	0.56
24	17.141	2-Hydroxy-1- (hydroxymethyl) ethyl palmitate	$C_{19}H_{38}O_4$	330	0.76
25	18.023	2-hydroxy-1 (hydroxymethyl) octadec-9 (Z)-enoate	$C_{21}H_{40}O_4$	356	2.41
26	19.281	Olean-12-en-3-one	$C_{30}H_{48}O$	424	7.86
27	19.875	Lupeol	$C_{30}H_{50}O$	426	37.53
28	20.812	Triamcinolone acetonide	$C_{24}H_{31}FO_6$	434	0.88

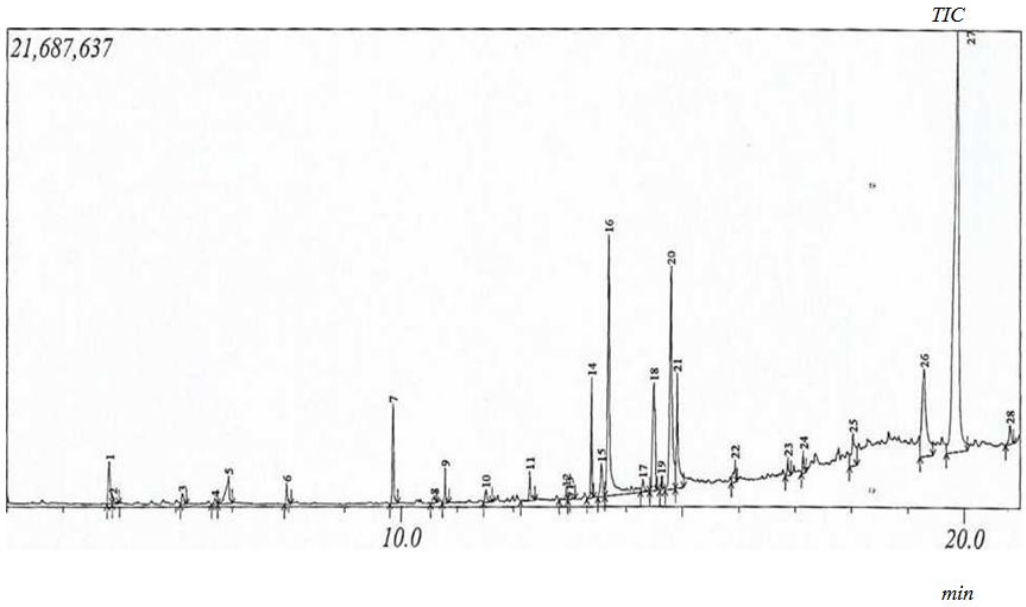


Fig. 1. Gas chromatography- mass spectrometry chromatogram of dichloromethane-methanol extract of *Garcinia kola fruit pulp*

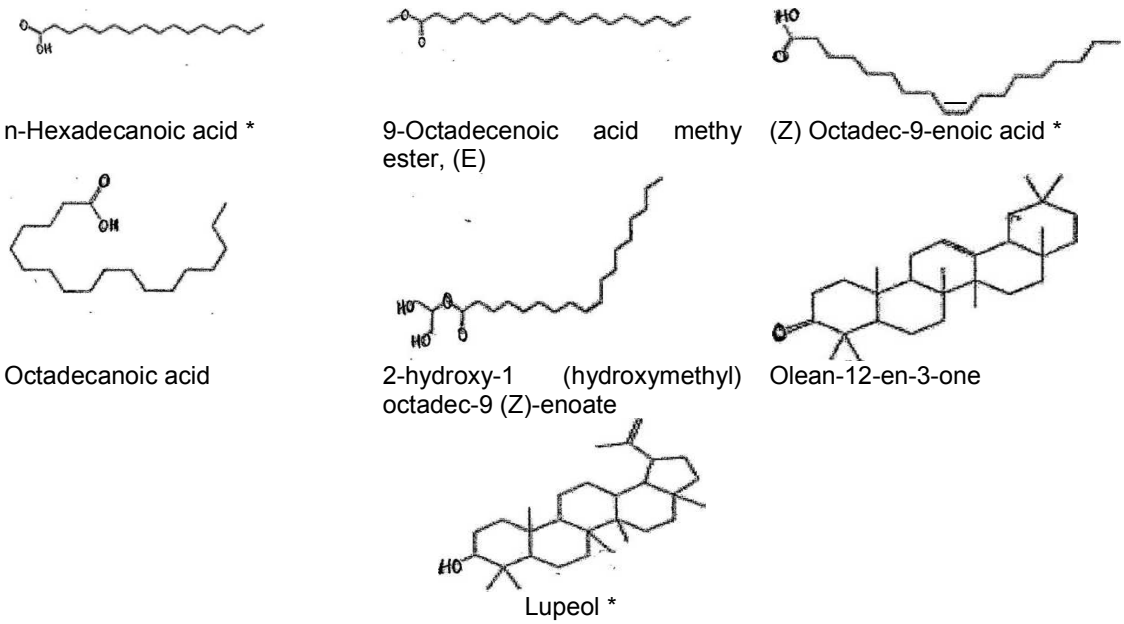


Fig. 2. Structures of compounds found in appreciable quantities in dichloromethane/ methanol extract of *Garcina kola* fruit pulp, (* refers to the most abundant bioactive compounds)

honey and milk as well as monitoring heating processes applied to cereal products such as pasta, during bread baking, bread slice toasting and extrution of baby cereals and breakfast cereals [24]. This suggests that 5-hydroxymethyl furfural identified in *G. kola* fruit pulp was generated during oven drying treatment. There has been controversial conclusions on the biological activity indicating it to be potentially carcinogenic to humans [24]. However, recent research has shown some positive effects such as being a novel natural antioxidant with potential applications in cancer chemoprevention [22], modifies intracellular sickcle hemoglobin and

inhibits sickling of red blood cells via Schiff base adducts with Hbs without inhibiting plasma and tissue proteins [21]. It is also reported to have cytoprotective effect by protecting normal liver cell line (L02) against cytotoxicity induced by hydrogen peroxide [25]. Currently under the development code Aes-103, 5-hydroxymethyl furfural has been considered for the treatment of sickle cell diseases [26]. 3-methyl-2,5-furandione has been reported to have anti-cancer activity [27].

Mostly identified compounds were ketone, fatty acids, fatty acid esters, sterols, flavonoids, triterpenoids, nitrogen compounds. The polarity of the respective solvents played a major role to extract more specific bioactive compounds. This was confirmed by the isolation of phyto-compounds on polarity based extraction [28]. Dichloromethane/methanol extracted more of the fatty acids and triterpenoids than methanol which extracted more of flavonoids, aldehydes and other water soluble compounds. It was observed that some compounds were common to both dichloromethane/methanol and methanol extract of *G. kola* fruit pulp but in varied concentrations. These include 4-methyl itaconate, 5-hydroxymethyl furfural, 4,5-diethenyl-2,2-dimethyl-1,3-dioxolane, trimethyl citrate , cis-9-hexadecenoic acid, n-Hexadecanoic acid, (Z) Octadec-9-enoic acid and octadecanoic acid.

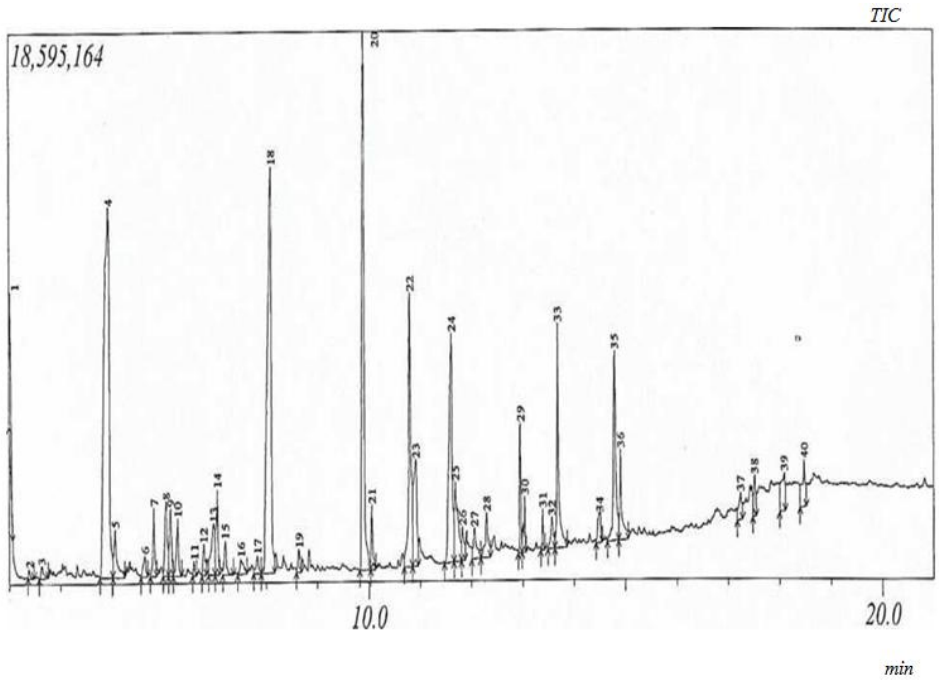
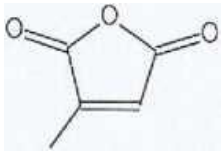


Fig. 3. Gas chromatography- mass spectrometry chromatogram of methanol extract of *Garcinia kola* fruit pulp

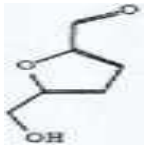
Table 2. Compounds identified in methanol extract of *garcinia kola* fruit pulp

Peak	RT	Name of compound	Molecular formula	Molecular weight	Relative abundance (%)
1.	3.023	Furfural	$C_5H_4O_2$	96	1.70
2.	3.385	4,4,5-Trimethyl-1,3-Dioxan-5-ol-	$C_7H_{14}O_3$	146	0.12
3.	3.628	Ethyl octyl ether	$C_{10}H_{22}O$	158	0.31
4.	4.934	3-Methyl-2,5-furandione	$C_5H_4O_3$	112	17.37
5.	5.060	1-Nitrosoazonane	$C_8H_{16}N_2O$	156	1.43
6.	5.640	4-Methoxy-4-oxobutanoic acid	$C_5H_8O_4$	132	0.55
7.	5.810	Methyl 2-furoate	$C_6H_6O_3$	126	1.28

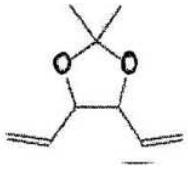
Peak	RT	Name of compound	Molecular formula	Molecular weight	Relative abundance (%)
8.	6.053	4H-Pyran-4-one- 2,3-dihydroxy-6-methyl	$C_6H_8O_4$	144	1.80
9.	6.139	2(3H)-Furanone, 5-ethenyldihydro-5-methyl	$C_7H_{10}O_2$	126	1.38
10.	6.281	Methyl 2,5-dihydro-1H-pyrrole-2-carboxylate	$C_6H_9NO_2$	127	1.23
11.	6.596	Dimethyl malate	$C_6H_{10}O_5$	162	0.20
12.	6.785	Levoglucosenone	$C_6H_6O_3$	126	0.60
13.	6.978	4-Methyl itaconate	$C_6H_8O_4$	144	1.72
14.	7.050	4-Methyl itaconate	$C_6H_8O_4$	144	1.67
15.	7.021	1,3-propanediol, 2-ethyl-2(hydroxymethyl)	$C_6H_{14}O_3$	134	0.68
16.	7.503	3,7-Dimethyloct-6-en-1-yl 2-methylbutanoate	$C_{15}H_{28}O_2$	240	0.54
17.	7.825	6-Methyl-3-isopropyltetrahydro-2H-pyran-2-one	$C_9H_{16}O_2$	156	0.42
18.	8.096	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	14.53
19.	8.637	3,4,5,6,7,8-Hexahydro-2H-chromene	$C_9H_{14}O$	138	0.50
20.	9.902	4,5-diethenyl-2,2-dimethyl-1,3-dioxolane	$C_9H_{14}O_2$	154	10.55
21.	10.057	2-Furanmethanol	$C_5H_6O_2$	98	0.90
22.	10.805	Trimethyl citrate	$C_9H_{14}O_7$	234	6.01
23.	10.918	1,6-Anhydro-β-D-glucose	$C_6H_{10}O_5$	162	3.45
24.	11.608	2-Hydroxypropane-1,2,3-tricarboxylic acid, dimethyl ester	$C_8H_{12}O_7$	220	6.61
25.	11.685	Undecanoic acid	$C_{11}H_{22}O_2$	186	2.57
26.	11.824	1-methyl-1-(2-tridecyl)oxy-1-silacyclopentane	$C_{18}H_{38}OSi$	298	0.72
27.	12.054	(+)-4a,7,7a,8,9,10,11a-Octahydro-3,3,7,10,11-pentamethyl-6-oxo-1,2,4-trioxino(6.5j)-2-benzopyran	$C_{15}H_{24}O_5$	284	0.94
28.	12.301	n-Tetradecanoic acid (Myristic acid)	$C_{14}H_{28}O_2$	228	1.35
29.	12.955	2,10-Dimethyl-9-undecen-1-ol	$C_{13}H_{26}O$	198	1.51
30.	13.035	2,10-Dimethyl-9-undecen-1-ol	$C_{13}H_{26}O$	198	0.87
31.	13.383	Methyl 14-methylpentadecanoate	$C_{17}H_{34}O_2$	270	0.39
32.	13.563	Cis-hexadec-9-enoic acid	$C_{16}H_{30}O_2$	254	0.76
33.	13.683	n-Hexadecanoic acid.	$C_{16}H_{32}O_2$	256	4.03
34.	14.492	α.-Glyceryl linolenate	$C_{21}H_{36}O_4$	352	0.79
35.	14.789	(Z)-Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	4.28
36.	14.903	Octadecanoic acid	$C_{18}C_{36}O_2$	284	1.88
37.	17.230	(3β, 17β)-Spiro [androst-5-ene-17,1^1-cyclobuta]2^1-one,3-hydroxy	$C_{22}H_{32}O_2$	328	0.76
38.	17.506	Cinnamic acid, 4-hydroxy-3-methoxy-,{5-hydroxy-2-hydroxymethyl-6-[2-(4-hydroxy-3-methoxyphenyl)ethoxy)-4-(6-methyl-3,4,5-trihydroxytetral	$C_{31}H_{40}O_{15}$	652	0.81
39.	18.081	(3β,5α,22E)-Stigmasta-7,22-diene-3-yl,acetate	$C_{31}H_{50}O_2$	454	1.37
40.	18.469	(1,1^1-Bicyclopropyl)2-octanoic acid 2^1-hexyl-methyl ester	$C_{21}H_{38}O_2$	322	1.42



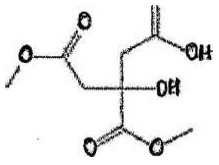
3-Methyl-2,5-furandione *



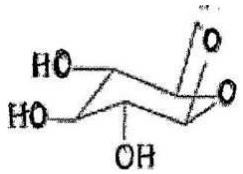
5-Hydroxymethylfurfural*



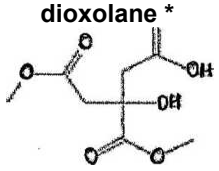
4,5-diethenyl-2,2-dimethyl-1,3-dioxolane *



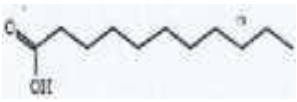
Trimethyl citrate



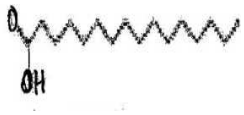
1,6-Anhydro-β-D-glucose



2-Hydroxypropane-1,2,3-tricarboxylic acid, dimethyl ester



Undecanoic acid



Hexadecanoic acid

(Z)-Octadec-9-enoic acid

Fig. 4. Structures of compounds found in appreciable quantities in methanol extract of *Garcina kola* fruit pulp, (*refers to the most abundant bioactive compounds)

4. CONCLUSION

In the present study 38 compounds from methanol extract and 28 compounds from dichloromethane/methanol extract were identified in *Garcinia kola* fruit pulp. The polarity of solvents used for extraction resulted for variations in bioactive compounds extracted. Lupeol (triperpene) was the major bioactive compound from dichloromethane/ methanol extract while 3-Methyl-2,5-furandione (flavonoid) was found to be the most abundant in the methanol extract of *G. kola* fruit pulp.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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