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Isolation and Phytochemical Characterization of Bioactive Constituents from the Seeds of *Garcinia kola*, Heckel (Clusiaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMZ conceptualized and designed the experiment as well as drafting of the manuscript. Authors AMZ and UHD carried out the experiments and interpretation of data. Author AN managed the literature searches and typesetting of the manuscript. Author ABS provided intellectual guide in the design of the experiment. Authors SS, SMH and GI revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out with the aim of isolating and identifying the bioactive constituents of the ethyl acetate extract from *G. kola* seeds that was a strong inhibitor of α-glucosidase using FT-IR and GC-MS techniques.
Place and Duration of Study: The study was carried out at Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria between October – December 2014.
Methodology: n-Hexane, ethyl acetate and methanol extracts were prepared gradient wise in a soxhlet apparatus at 50ºC. Column chromatographic analysis was carried out on the ethyl acetate extract. The isolate was purified and the structure elucidated by Fourier Transformed Infra Red spectroscopy and Mass Spectrophotometry.

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Results: Column chromatographic analyses and purification of column fraction B of the ethyl acetate extract lead to the isolation of ZAAK. Fourier Transformed-Infra Red spectra revealed the presence of carboxylic acid and an ester in ZAAK. Gas Chromatography revealed three major peaks with retention times at 17.99 min, 20.83 min and 21.08 min, thus suggesting that ZAAK is a mixture of three compounds and were subsequently labeled $ZAAK_1$, $ZAAK_2$, and $ZAAK_3$. The mass spectra identified $ZAAK_1$ $ZAAK_2$ and $ZAAK_3$ as 1-pentadecanecarboxylic acid, (Z)-11-Octadecenoic acid and octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester respectively.
Conclusion: On the basis of column chromatography and spectroscopy, ZAAK was identified as a mixture of fatty acids and fatty acid ester.

Keywords: Garcinia kola; gas chromatography; mass spectra; FT-IR.

1. INTRODUCTION

Since time in-memorial, medicinal plants have been constantly explored in search of new drugs with curative properties to treat various diseases. The biological potency of medicinal plants in the form of secondary metabolites has been known to be responsible for such properties. In developing countries such as Nigeria, traditional medicine practice still relies heavily on the use of plants. Today, a substantial number of drugs are developed from medicinal plants which are active against a number of diseases majority of which involves isolation of the active ingredient and its subsequent modification [1,2].

Garcinia kola is an angiosperm belonging to the family Clusiaceae, found growing predominantly in the southeast and southwestern states of Nigeria. It is locally called “Aku ilu” in Igbo, “Namijin goro” in Hausa and “Orogbo” in Yoruba [3]. Traditionally, the seed is used in the treatment of cough, throat infections, bronchitis, purgation, wound healing, jaundice, liver disorders and stomach upset [4-6]. Some reported pharmacological activities include: antimicrobial, antioxidant, antidiabetic, hepatoprotective and antipyretic activities [7-11]. Chemical investigations on the seed resulted in the isolation Cycloartenol and its 2, 4-methylene derivative [12], Garcinia biflavonoid 1, 2, Kolaflavanone [13], Apigenin 5, 7, 4′ – trimethylether, Fisetin [14], Garcioic acid, garcinal, and σ-tocotrienol [15].

In recent years, there has been an increase in the search for food grade α-amylase and α-glucosidase inhibitors from dietary plant extracts with lesser side effects to manage postprandial hyperglycemia. Zakariya and co-workers reported the inhibitory activity of *G. kola* seed extracts on carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) [16]. Therefore, this study is aimed at isolation and identification of bioactive constituents of ethyl acetate extract from *G. kola* seeds that was a strong inhibitor of α-glucosidase using FT-IR and GC-MS techniques.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Garcinia kola seeds were purchased from Sabon Gari market in Sabon Gari Local Government Area (Lat. 11º 09' N, Long. 7º 41' E) of Kaduna State and authenticated at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University Zaria (Lat. 11º 11' N, Long. 7º 38' E). The seeds were cut into smaller pieces and shade dried for about 14 days. They were then pulverized into powder using mechanical grinder.

2.2 Preparation of Extract from *Garcinia kola* Seed Powder

The solvents used during the course of this study were of analytical grade. Powdered sample (430 g) was extracted with n-hexane (Loba Chemic, India), ethyl acetate (Sigma Aldrich, USA) and methanol (Sigma Aldrich, USA) gradient wise in a soxhlet apparatus. The plant material was exhaustively extracted with hexane (1 L) until the solvent became clear and the same procedure was applied consecutively to ethyl acetate and methanol. The gradient extracts obtained with each solvent were concentrated on a rotary evaporator and finally dried to a constant weight after which it was stored in an air-tight container for subsequent use [17] with modification.

2.3 Column Chromatographic Analysis

The adsorbent, silica gel (100 g, 60-120 µm) was carefully packed using wet slurry method. Ethyl acetate extract, 3 g was loaded on to packed

adsorbent and allowed to stabilize. Chloroform (JHD, China), 100% was used as initial eluent followed by addition of ethyl acetate gradient wise. Volumes of 20 ml each were collected per fraction and allowed to concentrate under room temperature. Volume total of 260 ml equivalent to 13 fractions were eluted through the column for each solvent ratio. A total of 82 fractions were collected from the column. The fractions collected were monitored on TLC plates and visualized with 10% H_2SO_4 in methanol as spraying reagent. Similar fractions were pooled together and coded [18,19].

2.4 Isolation and Purification of ZAAK

Fractions 30-36 eluted from column with Chloroform: Ethyl acetate (9:1) after 680 ml of solvents elution through the column were pooled together and coded B weighing 213 mg. The pooled fraction (B) was purified by preparative TLC (PTLC) developed in Chloroform: Ethyl acetate (9:1) and Hexane: Ethyl acetate (7:3). Developed plates were scraped, washed and observed on TLC (Merck F_{254}) for the appearance of a single spot which was then coded ZAAK.

2.5 Fourier Transformed Infra-Red Spectroscopy of ZAAK

The isolate ZAAK was mixed with 5 mg of KBr and ground to a very fine powder. The powder was compressed under high pressure using a press to produce pellets. The pellets were then analyzed on FTIR-8400S Fourier transform infrared spectrophotometer. The bands were compared with those reported in literatures.

2.6 Gas Chromatography-Mass Spectrophotometry of ZAAK

Gas Chromatography-Mass Spectrometry (GC-MS) was carried out on the isolate, ZAAK on a GC-MS-QP2010 plus SHIMADZU with SGE BPX5 column (30 m × 0.25 mm, I.D × 0.25 µm). Oven temperature was set at 80℃ to 280℃ at 30℃/min. Injection temperature was set at 250℃ using a split mode. Helium gas (99.9%) was used as the carrier. The flow rate of helium gas was 1.58 ml/min with a total GC-MS running time of 27 minutes. The ionization mass spectroscopic analysis was done with 70 eV. Mass spectra were recorded across the range of 40 to 600 m/z for the duration of 28 min. Peak areas were compared with the database in the GC-MS library version NIST 05-S and reported literatures.

3. RESULTS AND DISCUSSION

3.1 Results

Column chromatography of the ethyl acetate extracts lead to the isolation of a colorless amorphous substance weighing 23 mg after preparative TLC, soluble in chloroform and ethyl acetate. The isolate was labeled ZAAK.

Fourier-Transformed Infra Red (FT-IR) spectra of ZAAK showed υ_{max} (KBr): 3424.73 cm^{-1} (−OH stretching), 2933.83 cm^{-1} (−CH_2 stretching), 2872.10 cm^{-1} (−CH_3 stretching), 1731.17 cm^{-1} and 1608.69 cm^{-1} (−C=O stretching), 1040.63 cm^{-1} (−C-O-C), 1175.65 cm^{-1} (−C(=O)−C stretching), 914.29 cm^{-1} (−OH bending), 1452.45 cm^{-1} and 1373.36 cm^{-1} (−CH_3 bending) and 740.69 cm^{-1} (−CH_3 bending).

Total Ion Chromatogram (TIC) of ZAAK revealed three major peaks at retention times (t_R) 17.99, 20.83 and 21.08 minutes. These peaks were subsequently coded $ZAAK_1$, $ZAAK_2$ and $ZAAK_3$ respectively.

Mass spectrum of $ZAAK_1$ showed a molecular ion peak $[M]^+$ at m/z 256, contributed 27.09 % to the TIC. Prominent fragment ions were observed at m/z 41, 55, 57, 60 and 73 with m/z 43 having the highest relative abundance being the base peak. Characteristic ion peaks at m/z 59, 71, 85, 99, 113, 115, 127, 129, 143,157, 171, 185, 199, 213 and 227 were also observed (Fig. 1).

The peak corresponding to $ZAAK_2$ revealed a molecular ion peak $[M]^+$ at m/z 282. Major peaks at m/z 41, 43, 67, 69, 83 and 97 were observed. The base peak was at m/z 55 with other fragment ions at m/z 125, 111, 85, 73, 71, 70, 59, 57 and 45 (Fig. 2). The percentage area peak was recorded as 45.33% of TIC.

A molecular ion peak $[M]^+$ at m/z 327 was recorded for $ZAAK_3$ (Fig. 3). Intense ion peaks at m/z 43, 55, 56, 57, 60 69, 71, 73 and 97 with m/z 41 as the base peak. Other important fragment ions were observed and recorded at m/z 45, 57, 59, 85, 87, 99, 101, 113, 115, 127, 129 and 143. A percentage area peak of 14.17 % was contributed to the TIC by $ZAAK_3$.

3.2 Discussion

Chemical screening is performed to target isolation of new or useful type of constituents having potential pharmacological activities. These procedures have been found to enable

recognition of known metabolites in plant extracts in the earliest stages of separation and thus economically very important [19]. Zakariya et al. [16], Confirmed the presence steroids/ terpenoids, phenolic compounds and flavonoids in the extract. These compounds support the basis for the reported inhibitory activity of *G. kola* seeds on carbohydrate hydrolyzing enzymes and necessitated the need for this study.

Fourier transformed infrared spectra revealed strong band at 2933.83 cm^{-1} corresponding to an asymmetrical methylene ($-CH_2$) stretching vibration indicating the presence of an aliphatic compound [20]. The band corresponding to C-H bending of methylene rocking vibration in which all of the methylene groups rock in phase occurred at 740.69 cm^{-1} characteristics of a straight chain hydrocarbon of seven or more carbon atoms [20,21]. Absorption band at 1040.63 cm^{-1} revealed symmetrical −C-O-C stretching vibrations indicative of an alkyl ether group. Carbonyl carbon (−C=O) stretching vibration for monomers of saturated aliphatic carboxylic acids was recorded at 1608.69 cm^{-1}. A shift in this band from 1720-1706 cm^{-1} to a lower frequency could be due to internal hydrogen bonding occurring in the molecule. Internal hydrogen bonding reduces the frequencies of −C=O stretching absorption to a greater degree than does the intermolecular hydrogen bonding as observed in salycylic acid (1665 cm^{-1}) and para-hydrobenzoic acid (1680 cm^{-1}) [21]. Hydroxyl (−OH) bending vibration of the bonded −OH is an important characteristic band in the spectra of dimeric carboxylic acid [21]. Hence the band at 914.29 cm^{-1} is suggestive of dimerized carboxylic acid. A broad band (−OH) stretching recorded at relatively higher frequency, 3424.73 cm^{-1} that may be due to stearic hindrance is suggestive of the presence of an isolated −OH group [20]. Carbonyl (−C=O) stretching for ester recorded at 1731.17 cm^{-1} indicating a saturated aliphatic ester.

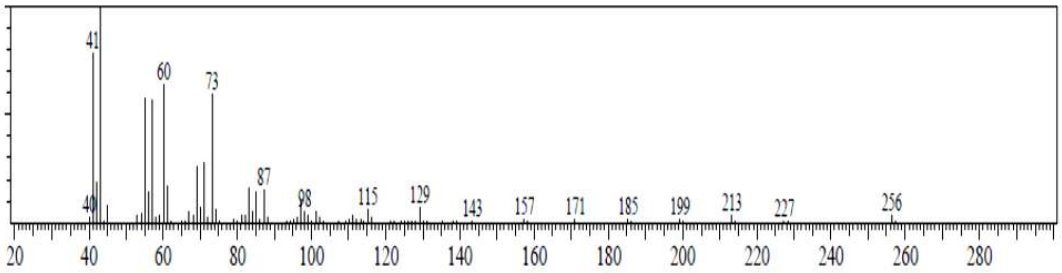


Fig. 1. Mass spectra of $ZAAK_1$

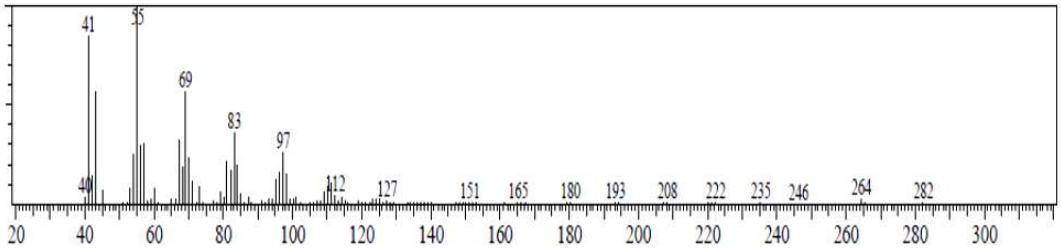


Fig. 2. Mass spectra of $ZAAK_2$

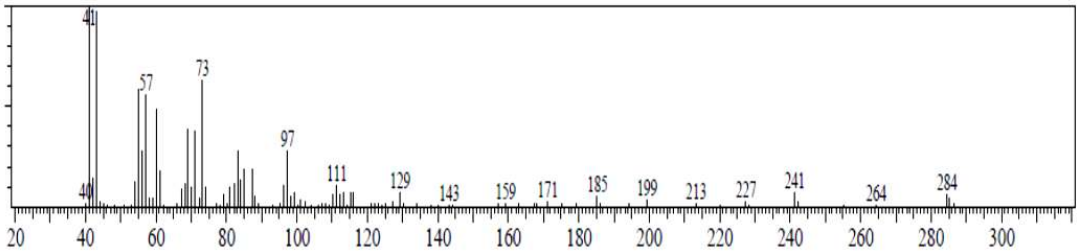
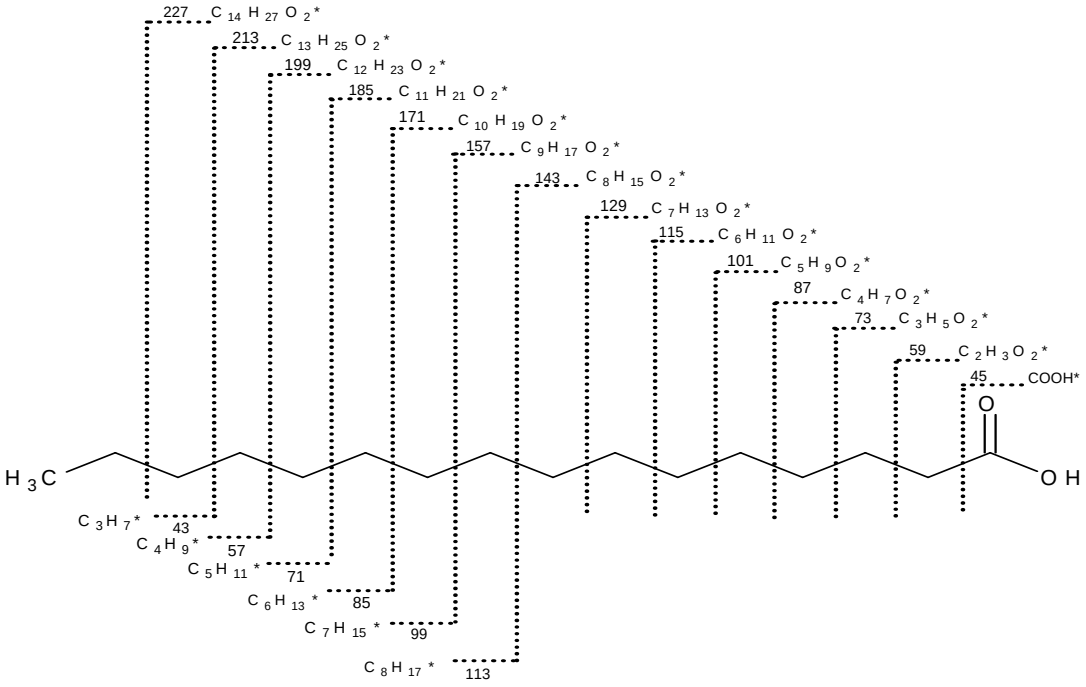
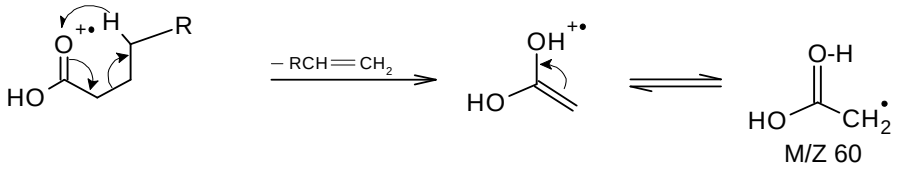


Fig. 3. Mass spectra of $ZAAK_3$



Scheme 1a. Schematic representation of mass fragmentation pattern for spectral peak of $ZAAK_1$



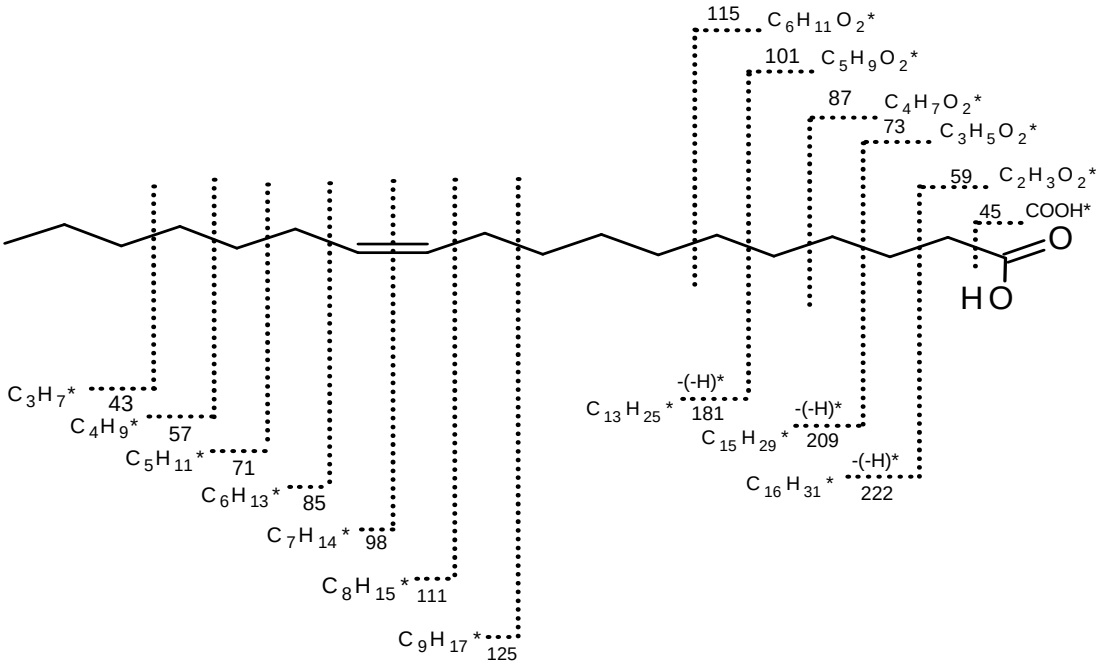
Scheme 1b. Mechanism of McLafferty rearrangement of compound $ZAAK_1$

The mass spectra of $ZAAK_1$ showed a molecular ion $[M]^+$ at m/z 256 (Fig. 1). The molecular ion peak represents the intact molecule and also gives the exact molecular weight of the compound [22]. Ion peak at m/z 45 indicates loss of –COOH, characteristics of carboxylic acids. An intense ion peak at m/z 60 (Fig. 1) that may have resulted from the intra-molecular γ-hydrogen transfer to the ionized carbonyl oxygen via the hexacyclic transition state (McLafferty rearrangement) followed by Cα−Cβ bond cleavage [23] (Scheme 1b) is diagnostic for straight chain carboxylic acids. In straight chain monocarboxylic acids, the most characteristic and sometimes the base peak is m/z 60 [21]. Diagnostic peaks at m/z 43, 57, 71, 85, 99, 113 and 127 (Scheme 1a) are suggestive of alkyl ion fragment resulting from C−C bond cleavage. Oxygen containing fragment ions observed at m/z 45, 59, 73, 87, 101, 115, 129, 143, 157, 171, 185, 199, 213 and 227 (Scheme 1a) can be attributed to the formation of hydrocarbon clusters at an interval of 14 mass units giving by $C_nH_{2n-1}O_2$ [24]. In long chain carboxylic acids, the spectrum consist of two series of peaks resulting from cleavage at each C−C bond with retention of charge either on the oxygen containing fragment or on the alkyl fragment [21]. These ions together with a molecular ion at m/z 256 are suggestive of $C_{16}H_{32}O_2$ molecular formula called 1-pentadecanecarbxylic acid (palmitic acid). This data is in agreement with the library hit and reported literatures [25].

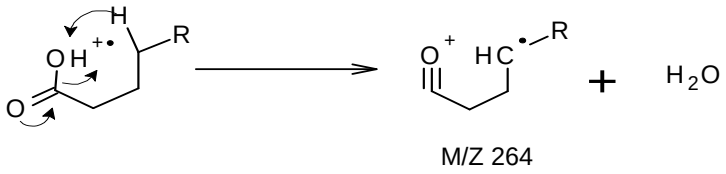
The fragment ion at m/z 264 for $ZAAK_2$ (Scheme 2b) is associated with the loss of 18 mass $[M-18]^+$ which is equivalent to mass of water molecule. The loss of water molecule suggests the presence of a hydroxyl (−OH) group. A rearrangement of hydrogen accompanied fragmentation, which is evident from the even-numbered fragment ions such as those at m/z

264, 222 and 180 (Fig. 2) arising from an even-numbered molecular ion [21]. The peaks at m/z 60 and m/z 45 are indicative of McLafferty rearrangement (Scheme 1b) and loss of –COOH (Scheme 2a), characteristic of carboxylic acids. Ion peaks at m/z 41, 55,69, 83 and 97 (Fig. 2) could be attributed to the loss in mass of 14 equivalent in mass to $-CH_2$ resembling the series of hydrocarbon clusters at interval of 14 mass units with each cluster having a prominent peak at $C_nH_{2n-1}O_2$. The difference of 13 amu between homologous fragmentation ions at m/z 111, C_{11} and m/z 98, C_{12} (Scheme 2a) can be associated to the presence of a double bond indicating Δ_{11} unsaturation. Other diagnostic peaks could be associated with the cleavage of the C−C bond giving alkyl ions and oxygen containing ions (Scheme 2a), a common feature of aliphatic carboxylic acids. This fragmentation pattern suggests the presence of an unsaturated fatty acid (18:1) with $C_{18}H_{34}O_2$ molecular formula called (Z)-11-octadecenoic acid (Vaccenic acid). This is in agreement with reported literatures [21,26].

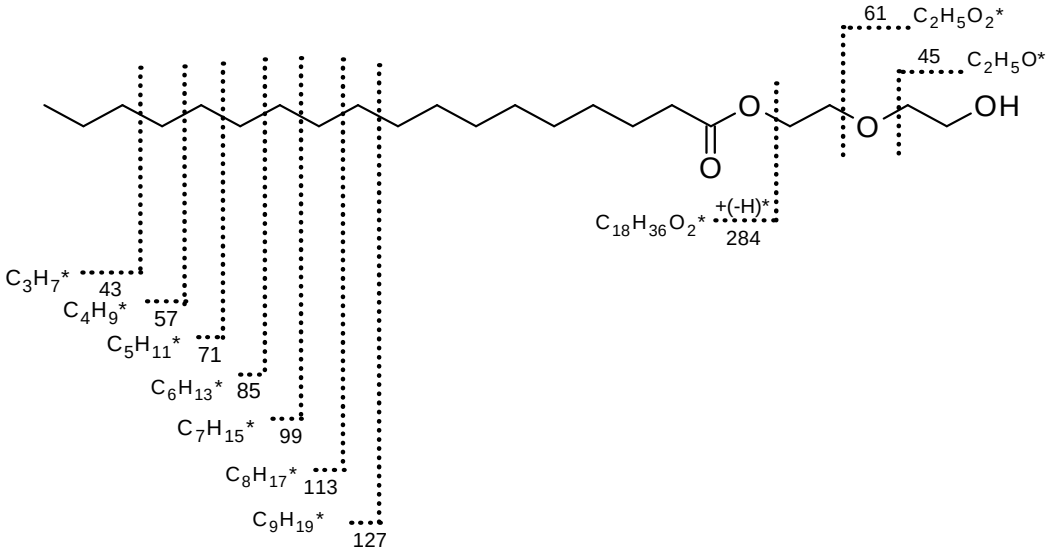
The intensity of a molecular ion peak depends on the stability of the molecular ion, for, if substituent's that have favorable modes of cleavage are present, the molecular ion peak will be less intense and the fragment peaks relatively more intense [21], these might have accounted for the less intense molecular ion peak of $ZAAK_3$. Characteristic fragment ion at m/z 60 could be associated with possible rearrangement and fragmentation of the acid portion of the molecule i.e. McLafferty rearrangement (Scheme 1b) [27]. Fragment ion at m/z 284 suggests loss of 89 mass an equivalent mass to $C_4H_9O_2$ (Scheme 3).



Scheme 2a. Schematic representation of mass fragmentation pattern for spectral peak of $ZAAK_2$



Scheme 2b. Mechanism of McLafferty rearrangement of compound $ZAAK_2$



Scheme 3. Schematic representation of mass fragmentation pattern for spectral peak of $ZAAK_3$

Fragmentation pattern for esters of straight chain carboxylic acids can be described in the same terms as for the free acid [21]. This may be responsible for the fragment ions at m/z 143, 129, 115, 101, 87, 73, 59, suggesting alkyl fragment ions and oxygen containing ions at m/z 41, 55, 69, 83, 97 and 111 (Fig. 3). Other fragment ions such as those at m/z 87 and 59 are diagnostic of aliphatic ether (Fig. 3). Therefore, this mass ionization pattern indicates a 372 molecular mass compound of $C_{22}H_{44}O_4$ formula, suggesting an octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ether (aqua cera). These mass features were consistent with computer data and reported literatures [24,28].

However, it is not clear if these fatty acids have contributing effect on the inhibition of α-glucosidase as reported by [16]. Although, fatty acids such as stearic acid from the seed of *Mormordica charantia* has been reported to have inhibitory effect on α-amylase and α-glucosidase, hence its antidiabetic property [29]. Palmitic acid has been reported to have hypoglycemic activity [30], antimicrobial activity [31,32], antioxidant and anti-inflammatory, hypocholestrolemic, haemolytic 5-alpha-reductase inhibitor [33,34]. Aqua cera is used in cosmetics and textile industries. They serve as plasticizers, lubricants, binding and thickening agent [35].

4. CONCLUSION

On the basis of column chromatography and spectroscopic studies (FT-IR and MS), palmitic acid, cis-Vaccenic acid and aqua cera were isolated as a mixture and characterized. These compounds are reported for the first time as constituents of *Garcinia kola* seeds. Palmitic acids have been associated with some pharmacological activities, it will be of no surprise if it contributes to ethyl acetate being a strong inhibitor of α-glucosidase, while, aqua cera could hold a lot of prospects in the pharmaceutical industries as a binding and thickening agent.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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