Chemistry Research Journal, 2017, 2(5):153-162

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Metabolites Composition of *Garcinia kola* Extract as Potential Substitute for Isomerized Hop Extract in Beer Brewing

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Abstract The potential of methanoilc extract of *Garcinia kola* (bitter cola) as substitute for isomerized hop extract in beer brewing was evaluated. The number of metabolites in each extract was investigated using Thin Layer Chromatography (TLC) technique. The relative proportion of each metabolite in the two extracts was investigated using Gas Chromatography - Mass Spectrometer (GC-MS). The TLC results revealed that the extract of G. kola contains 12 metabolites while isomerized hop extract contains 14 metabolites. The GCMS results were in agreement with the results of the TLC with respect to the number of metabolites in each extract. The results of the GCMS also showed that *Garcinia kola* extract contains some metabolites comparable to those of isomerized hop extract, although some metabolites [dehydro-cohumulunic acid; 4,4-dimethyl-2-buten-4-olide; 1,2-dimethyl-cyclopropane carboxylic acid; lupulone; 2,5-dimethyl-2-hexanol; 4,4,5,5-tetramethyl-bicyclo hexyl-6-ene-2,3-dione; octadecanoic acid, oxiranyl methyl ester and 1,2-benzenedicarboxylic, bis(-2-ethyl hexyl) ester] present in isomerized hop extract were absent in *Garcinia kola* extract. Consequently, academic activity in the area of mixtures/blends of extract of plant species which mimic isomerized hop extract is strongly recommended.

Keywords Metabolite, GCMS, Extract, Hop, *Garcinia kola*

Introduction

The Nigerian beer industry is a very vital component of Nigeria's non-oil sector and has largely contributed to economic growth in recent times. The country's growing youth population, with increased disposable incomes is the constant drive that increased beer consumption in Nigeria [1]. Reports have shown that annual beer consumption in Nigeria increased from 115 million hectoliter in 2008 to 151.5 million hectoliter in 2011 [2]. Therefore, beer production has increased both in brands and quantities recently due to ready markets. Consequently, the importation of hops to meet the demands of the already expanded brewing industry has continued to constitute a significant proportion of imports into the country.

Beer, a brewed beverage is made principally from malt (germinated barley and/or sorghum), hop, water and yeast. Hop plants are vital to the brewing industry and some of their unique chemicals have the potential to be used in the nutraceutical industry [3]. Hops, a minor ingredient in beer brewing, are used for their bittering, flavouring, and aroma enhancing powers. They also have pronounced bacteriostic activity that inhibits the growth of gram-positive bacteria in the finished beer, thereby extending the shelf life of the product [4-6]. The brewing value of hops is

found in hop resins and essential oils that are contained in lupulin glands of the female hop cone. These contain bitter resins and ethereal oils which supply bittering and aroma components of hops. Hop resins are the most valuable and most characteristic components of hops. They give beer its bitter taste, improve foam stability and act as antiseptic towards micro-organisms [7-8].

Garcinia kola is regarded as a wonder plant because every part of the plant (bark, leaf, root, wood, seed) has been found to be of medicinal importance. The medicinal importance of bitter cola is based mainly on the phytochemical components of the palnt. From its roots to its leaves, the plant is known to contain several phytochemicals noted for their medicinal importance [9]. *Garcinia kola* seed is believed to contain a wide spectrum of organic compounds such as flavonoids which confer on it some antimicrobial and antifungal actions against gram negative and gram positive micro-organisms. The biological activities of flavonoids include action against allergies, inflammation, free radicals and hepatoxins [10]. *Garcinia kola* seeds are also used in the treatment of diabetes, bronchitis and throat infections as well as treatment of liver disease and diarrhea [9][11]. In Nigeria, a cold water extract of the roots and bark with salt are administered to cases of bronchial asthma or cough, or vomiting [9]. The medicinal properties of bitter cola can be classified under purgative, antiparasitic and antimicrobial. The use of *G. kola* as hop substitute in tropical beer brewing has been investigated [12-14].

Some pioneer work by Okafor and Anichie (1983) [15] showed that leaves of the vegetable, *Gongronema latifolium* (*utazi*) have great potential as substitute for hops in tropical beer brewing. Ajebesone and Aina (2004) [12] characterized four bitter plants namely *Azadirachta indica* (neem), *Garcinia kola* (bitter cola), *Gongronema latifolium* (heckel) and *Vernonia amygdalina* (bitter leaf) as potential substitutes for hops in tropical beer brewing. The work of Ajebesone and Aina revealed that one thing common to all the four plants is that they are bitter, like hops, but thrive in tropical regions, unlike hops. In 2009, Shellie *et al.* [3] worked on varietal characterization of hop by GCMS analysis of hop cone in Australia and investigations on metabolite profiling of plant extracts have been reported in various literatures. For example, in 2001, Roessner et al. [16] showed that profiling metabolites in plant extracts allows comprehensive phenotyping of genetically or environmentally modified plant systems as reported by Shellie *et al*. Those authors went further to report that such studies drawn on simple extraction procedures have shown to be very robust [17] and have permitted wide-range high-throughput applications, such as phenotyping to diagnostic analyses in plants.

The current research is on metabolite profiling of both *Garcinia kola* extract and isomerized hop extract by GCMS analysis to study the chemical composition of the two extracts in order to ascertain the suitability of *G. kola* as a possible substitute for isomerized hop extract in the Nigerian beer industry.

Methods

Sample Preparation

The isomerised hop extract was prepared by Ritchies, Rolleston Road, England, United Kingdom and the seed of the *Garcinia kola* plant sample was milled and vacuum dried at 50^{o}C. Five hundred grams (500g) of the plant material thus prepared was stored in a dessicator for the rest of the experiment. Two hundred grams (200g) of the resulting powders were then used to obtain methanolic extract by steeping procedure.

Methanol Extraction

The methanol extract was prepared by steeping 200g of the dry powdered plant material in 500 cm^3 of methanol at room temperature in a tight fitting round bottom flask for forty eight hours. The mixture was filtered first through a Whatman filter paper (No. 42) and then through a sintered glass funnel. The filtrate was concentrated using a rotary evaporator with water bath set at 40^{o}C for 2 hours to obtain the extract. The extract was stored in amber coloured reagent polypropylene bottle in a deep freezer (Thermofrost, Mod.TR150S) at -5^{o}C for subsequent analysis.

Thin Layer Chromatography (TLC)

The plate was prepared by smearing 20% aqueous slurry of silica gel in water on a precleaned and dried chromatographic plate (3.5cm x 7.5cm). This was dried in an oven at 105^{o}C for 1 hour. At the distance of 1.5cm from the bottom of the thin layer plate was drawn a horizontal line with pencil (2H) at which position the extract was spotted. This was inserted into the chromatographic tank and 25ml of the mobile solvent (CH_3OH) was introduced carefully down the side of the tank. The sample was eluted for 45 minutes after which the plate was put into a big bell jar wherein the colour of the fractions were developed using iodine crystals.

GC-MS Technique

The method adopted was that employed by Shellie *et al*., 2004 [3] as described by Okafor, 2016 [18]. GCMS analysis was performed using a Shimadzu GCMS-QP2010 plus (Schimadzu Oceania, Japan). A 60m x 0.25mm id BPX – 35 capillary columns with 0.25µm film thickness was used. Helium was used as carrier gas at a head pressure of 241250Pa to provide an initial flow rate of 2ml/min. A 1µl spitless injection (230^{o}C, 1.5min) was used. The GC temperature gradient was 85^{o}C to 330^{o}C at the rate of 4^{o}C/min and held at 330^{o}C for 10 minutes. Full-scan mass spectra were collected from 85 to 550 mass/charge ratio at a data acquisition rate of10 spectra/second. The MS transfer line was held at 250^{o}C and the ion source temperature was 200^{o}C.

Deconvolution of Metabolites

GC-TOFMS is a benchmark approach for metabolomics data acquisition from chromatographic peaks [19]. The GC component provides excellent sensitivity and sufficiently high data density to permit the deconvolution of overlapping metabolite peaks. It thus exhibits the power of clearly differentiating two or more closely associated chromatographic peaks which are commonly found in metabolite chromatograms. In addition, the MS component displays capacity to analyze each eluted chromatographic peak and subject the mass spectra to comparative analysis using a well appointed metabolite library of simulated mass spectral information [20-21]. In the present investigation, a scanning mass spectrometer was used to obtain chromatograms for the samples. Spectrum matching is achieved by programming the soft ware to compare the chromatogram of the mass spectra to simulated library peaks. The GC chromatograms of isomerized hop extract and that of *Garcinia kola* extract are shown in Figs.1 and 2 respectively. The conditions used to obtain the chromatograms are presented as foot notes below the chromatograms.

There are 14 chromatographic peaks in the GC chromatogram of isomerized hop extract and each peak represents a metabolite. Line#: 1 is the mass spectrum of peak 1 in the chromatogram. The analysis of this peak by the MS is presented in Hit#: (1-5). In this presentation, the closest simulated peak pattern is Hit#: 2.The instrument gives the systematic name of the metabolite as 4, 4-dimethyl-2-buten-4-olide. This process is shown in Fig.3. Similarly, the other chromatographic peaks are analyzed and named accordingly. This procedure was applied to the chromatographic peaks of the extract of *G. kola*.

Results and Discussion

TLC Results

The isomerized hop extract and *G. kola* extract showed a total number of 14 and 12 components respectively in the TLC chromatograms. This result was further confirmed by the GC chromatograms in Figs. 1 and 2 respectively.

GCMS Results

Isomerized hop extract contained fourteen metabolites as shown in Fig. 1 with 6-octadecenoic acid having the highest proportion of 28.92%. 1,2-Benzendicarboxylic acid, bis (2-ethylhexyl) ester had the lowest relative proportion of 1.14%. The extract of *G. kola* contained twelve metabolites (Fig.2) and 6-octadecenoic acid was also highest with a proportion of 44.09 % and hexadecanoic acid was least in proportion with 0.69 %. The metabolites,

their formulae, Structures and relative proportions of isimerized hop extract and *G. kola* extract are shown in Tables 1 and 2 respectively.

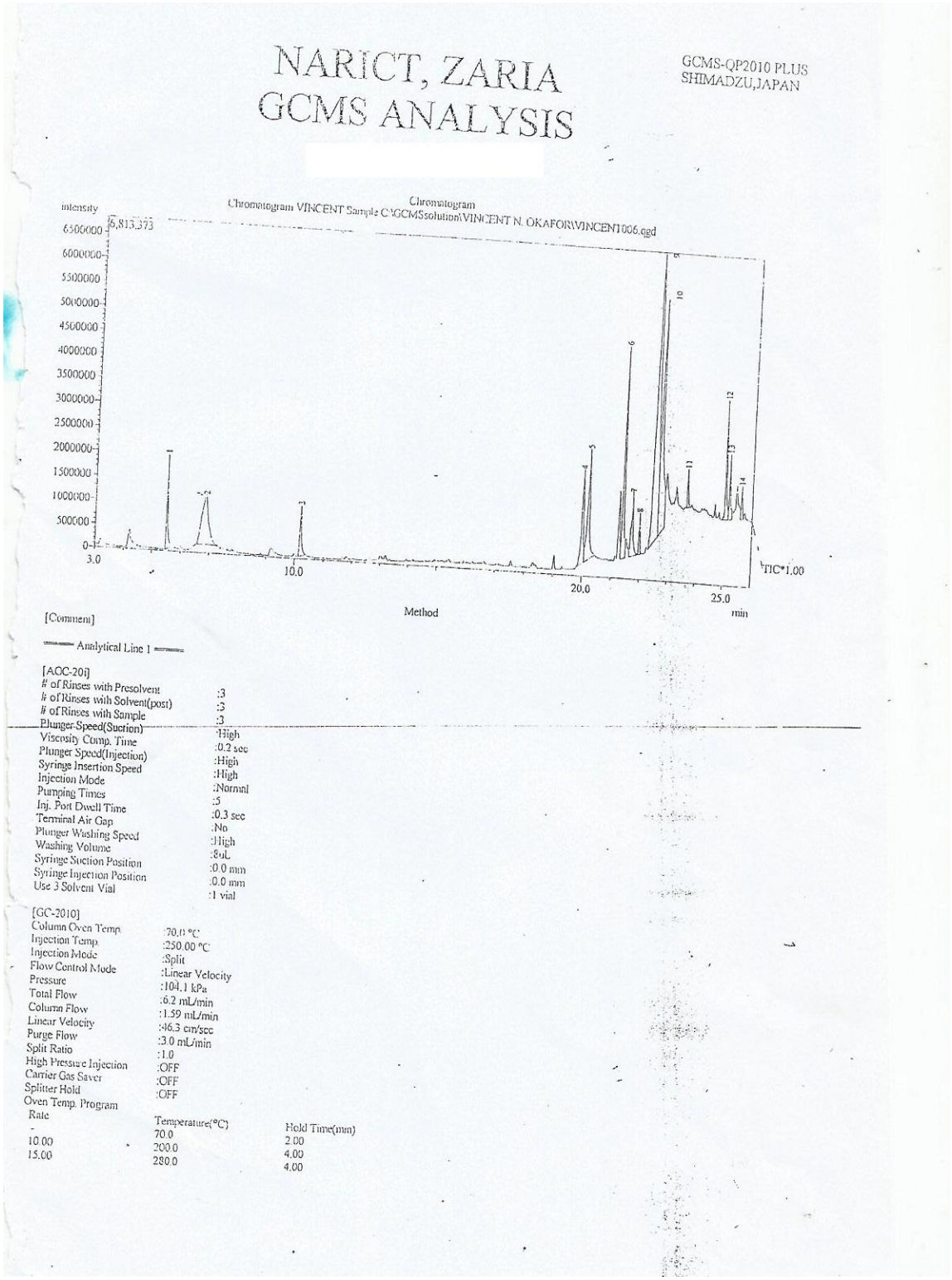
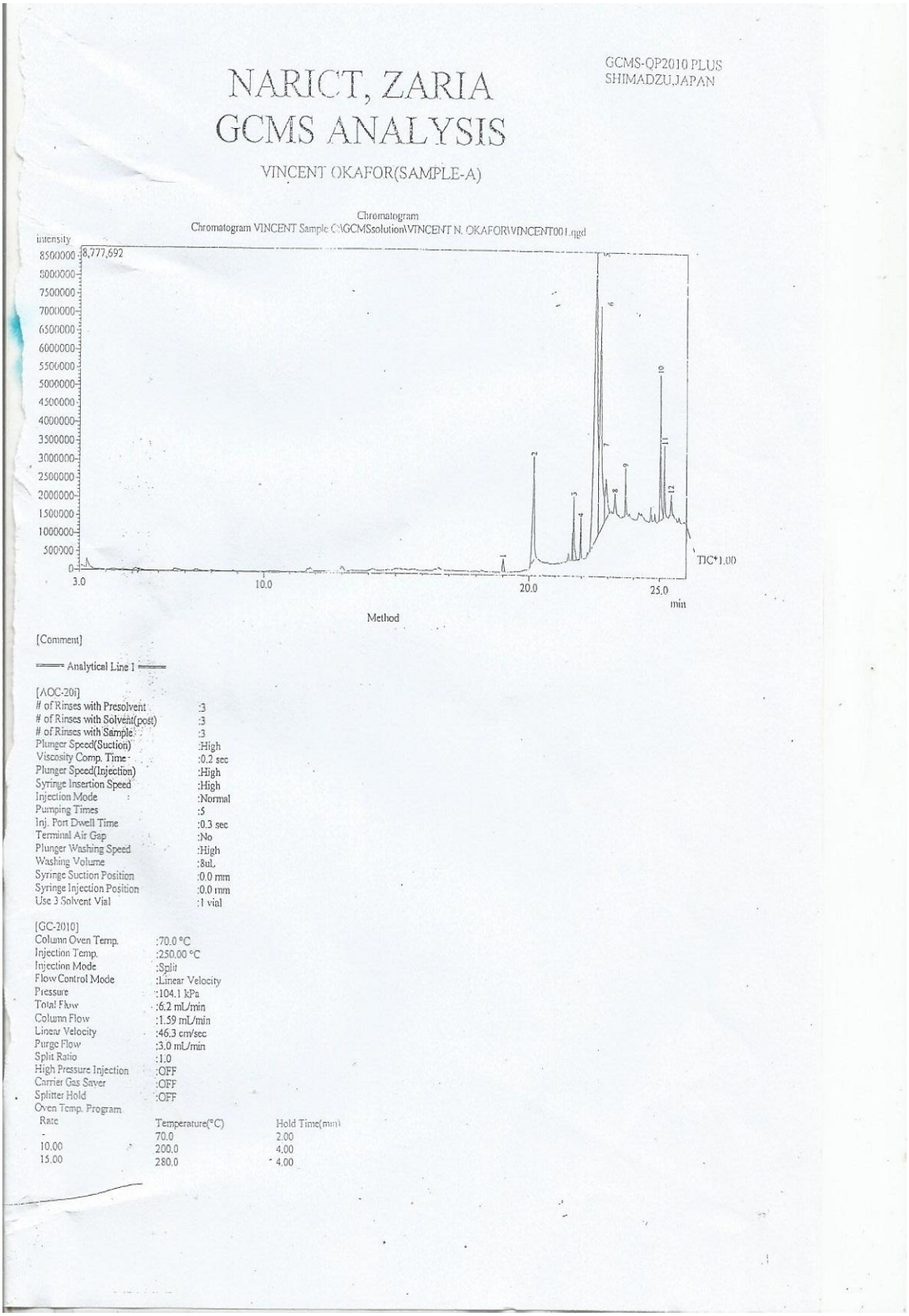


Figure 1: Chromatogram of isomerized hop extract



NARICT, ZARIA
GCMS ANALYSIS

GCMS-QP2010 PLUS
SHIMADZU,JAPAN

VINCENT OKAFOR(SAMPLE-A)

Chromatogram
Chromatogram VINCENT Sample C:\GCMSsolution\VINCENT N. OKAFOR\VINCENT001.qgd

Intensity
8,777,692
TIC*1.00
min

Method

[Comment]

Analytical Line 1

[AOC-20i]

# of Rinses with Presolvent	:3
# of Rinses with Solvent(post)	:3
# of Rinses with Sample	:3
Plunger Speed(Suction)	:High
Viscosity Comp. Time	:0.2 sec
Plunger Speed(Injection)	:High
Syringe Insertion Speed	:High
Injection Mode	:Normal
Pumping Times	:5
Inj. Port Dwell Time	:0.3 sec
Terminal Air Gap	:No
Plunger Washing Speed	:High
Washing Volume	:8ul.
Syringe Suction Position	:0.0 mm
Syringe Injection Position	:0.0 mm
Use 3 Solvent Vial	:1 vial

[GC-2010]

Column Oven Temp.	:70.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:104.1 kPa
Total Flow	:6.2 mL/min
Column Flow	:1.59 mL/min
Linear Velocity	:46.3 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:1.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	70.0	2.00
10.00	200.0	4.00
15.00	280.0	4.00

Figure 2: Chromatogram of Garcinia kola

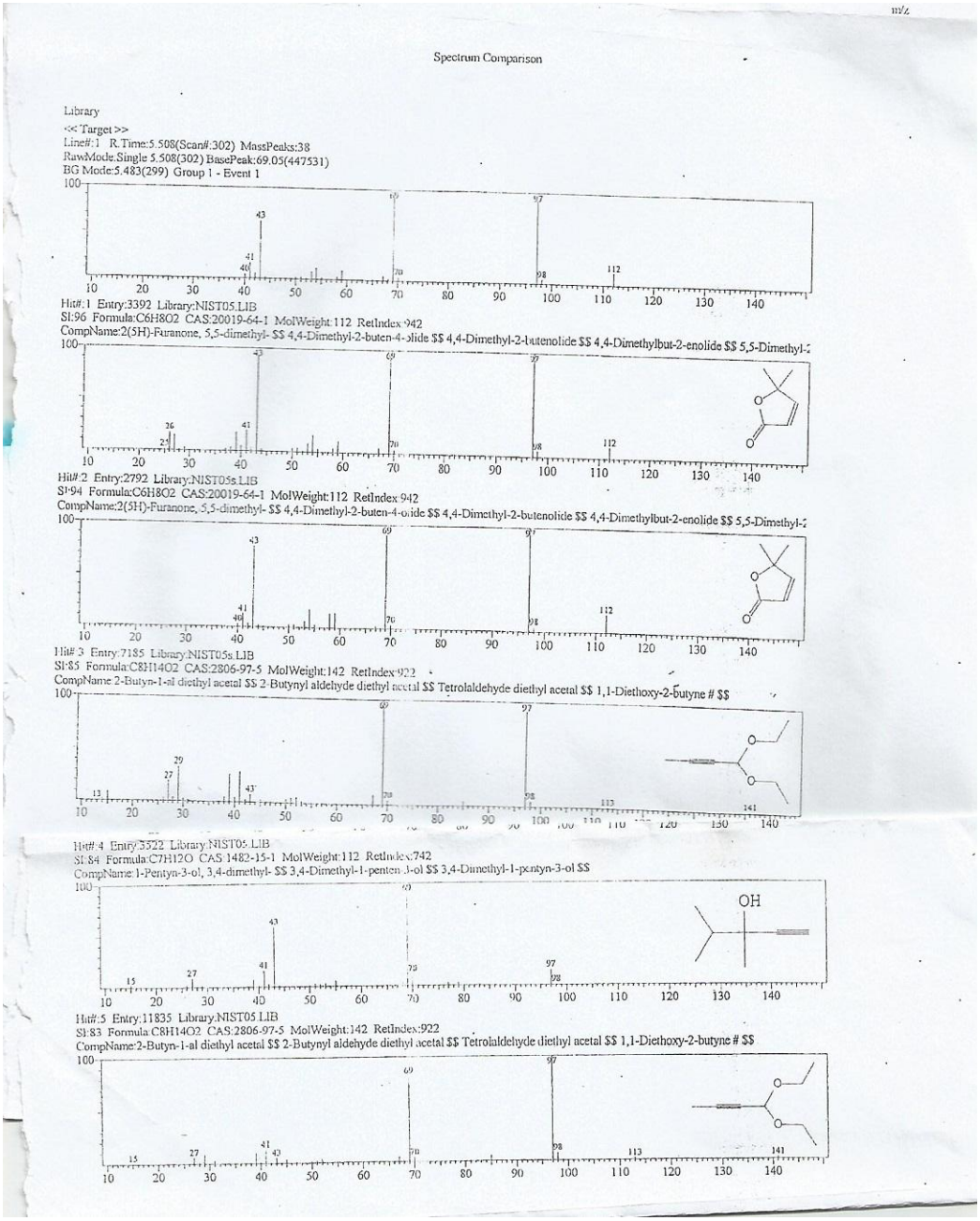


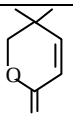
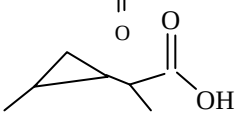
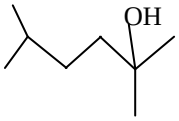
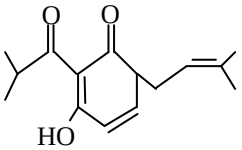
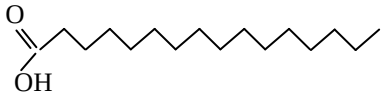
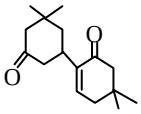
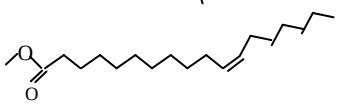
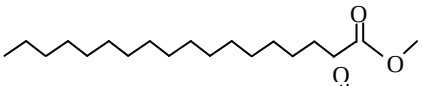
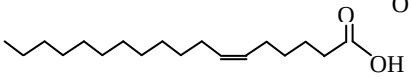
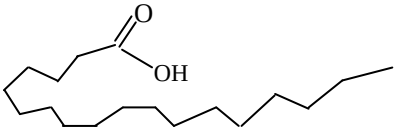
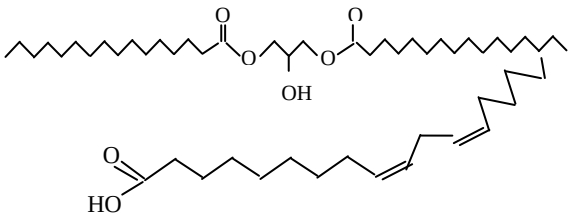
Figure 3: Spectrum comparison

There was presence of dehydro-cohumulunic acid, a derivative of an alpha acid called cohumulone in isimerized hop extract. Cohumulone generates isocohumulone by isomerization. Isocohumulones are chemical compounds that contribute to the bitter taste of beer and are in the class of compounds known as iso-alpha acids which contain approximately 40-80% bitter principles in the hop resin. The hop resin is known for its characteristic bitter taste in beer. There was also the presence of 9,12-octadecadienoic acid, the grape seed oil, which is an essential oil of the female hop cone responsible for flavouring and aroma enhancement in beers. All the other metaboltes however may

be responsible for other characters of hops e.g. pronounced bacteriostatic activity that inhibits the growth of gram-positive bacteria in the finished beer and precipitation of proteins

It is evident from Tables 1and 2 that 4,4-dimethyl-2-buten-4-olide ($C_6H_8O_2$); 1,2-dimethyl cyclopropane carboxylic acid ($C_6H_{10}O_2$); 2,5-dimethyl-2-hexanol ($C_8H_{18}O$); 4,4,5,5-tetramethyl bicyclo hexyl-6-ene-2,3,-dione ($C_{16}H_{24}O_2$); 1,2-benzen dicarboxylic acid bis (2-ethyl hexyl) ester ($C_{24}H_{38}O_4$) and dehydro-cohumulunic acid ($C_{14}H_{18}O_3$) were present in only isomerized hop extract while the extract of *G. kola* contains hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$), 9-hexadecenal ($C_{16}H_{30}O$), 2-methyl-3, 13-octadecadien-1-ol ($C_{19}H_{36}O$) and hexadecanoic acid, 2, 3-dihydroxypropyl ester ($C_{19}H_{38}O_4$) which were absent in isomerized hop extract.

Table 1: Relative Proportion of Metabolites of Isomerized Hop Extract

S/N	Metabolite	Formula	Structure	Relative Proportion (%)
1.	4,4-Dimethyl-2-buten-4-olide	$C_6H_8O_2$		3.62
2.	1,2-Dimethl cyclopropane carboxylic acid	$C_6H_{10}O_2$		9.90
3.	2,5-Dimethyl-2-hexanol	$C_8H_{18}O$		2.68
4.	Dehydro-cohumulinic acid	$C_{14}H_{18}O_3$		5.33
5.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		7.84
6.	4,4,5,5-Tetramethyl-bicyclo-hexyl-6-ene-2,3 dione	$C_{16}H_{24}O_2$		9.25
7.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		3.69
8.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		1.21
9.	6-Octadecenoic acid	$C_{18}H_{34}O_2$		28.96
10.	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$		17.92
11.	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$		1.24

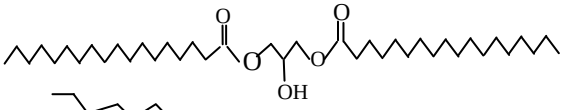
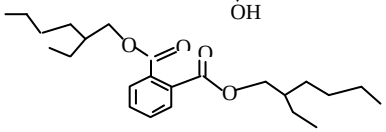
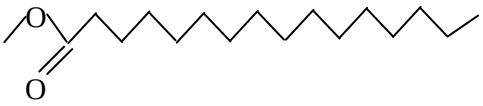
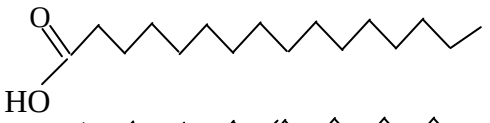
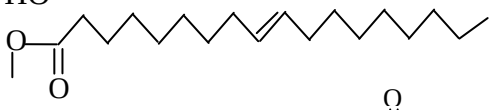
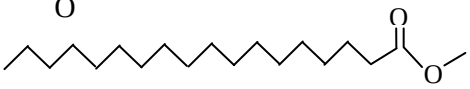
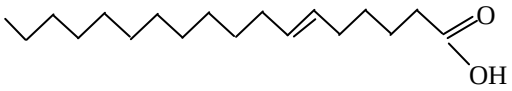
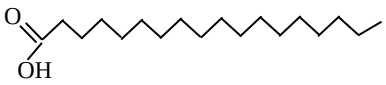
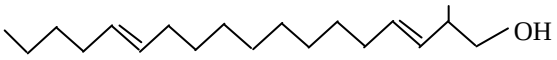
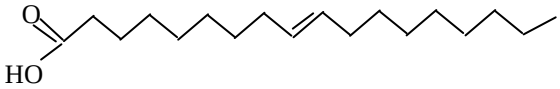
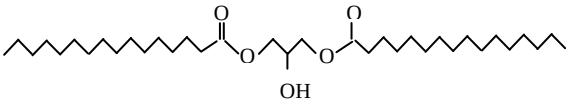
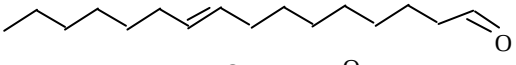
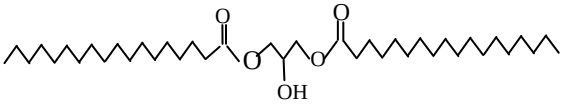
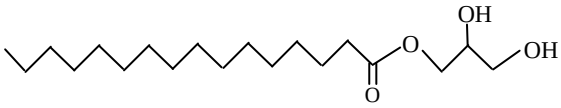
12.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$		4.65
13.	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$		2.57
14.	1,2-Benzendicarboxylic acid, bis (2-ethylhexyl) ester	$C_{24}H_{38}O_4$		1.14

Table 2: Relative Proportion of Metabolites of *G. kola* Extract

S/N	Metabolite	Formula	Structure	Relative Proportion (%)
1.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		0.69
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$		9.30
3.	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		2.84
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		1.62
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$		44.09
6.	Octadecanoic acid	$C_{18}H_{36}O_2$		23.31
7.	2-Methyl-3, 13-octadecadein-1-ol	$C_{19}H_{36}O$		4.23
8.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$		1.04
9.	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$		1.92
10.	9-Hexadecenal	$C_{16}H_{30}O$		7.07
11.	Octadecanoic acid, 2-hydroxyl-1, 3-propanediyl ester	$C_{39}H_{76}O_5$		2.84
12.	Hexadecanoic acid, 2, 3-dihydroxypropyl ester	$C_{19}H_{38}O_4$		1.04

It was also observed that both extracts contain hexadecanoic acid ($C_{16}H_{32}O_2$), octadecenoic acid methyl ester ($C_{19}H_{36}O_2$), Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester ($C_{35}H_{68}O_5$), octadecanoic acid methyl ester ($C_{19}H_{38}O_2$), 6-octadecenoic acid ($C_{18}H_{34}O_2$), octadecanoic acid ($C_{18}H_{36}O_2$), 9, 12-octadecadienoic acid, the grape seed oil ($C_{18}H_{32}O_2$) and octadecanoic acid, 2-hydroxyl-1, 3-propandiyl ester ($C_{39}H_{76}O_5$) in common. The results in this work are quite in agreement with those of Okafor, 2016 [18] in his book 'Hops and Potential Nigerian Substitutes'

This minor differences and major similarities in the constitution of metabolites in *G. kola* extract and isomerized hop extract is in agreement with the observation of Shellie *et al*. (2009) [3], in their varietal characterization of hop by GC-MS analysis of hop cone extracts and may explain the reason why the organoleptic character of beers brewed with imported hops and that of beers brewed with *G. latifolium* by Okafor and Anichie (1983) [15] were more pronounced while their chemical properties did not differ much. Furthermore, another interesting observation is that the relative proportion of metabolites which were commonly present in the two extracts are comparatively similar, example, the relative proportion of 6-octadecenoic acid is highest in proportion compared to the other metabolites in respective extracts. These results are consistent with results reported by Okafor *et al*. 2016 [22] in their characterization of hop extracts and extracts from four selected tropical plants by GCMS and brewing qualities analyses.

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