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Research Article

Gas Chromatography-Mass Spectrometry (GC-MS) Profiling of Hops and Some Nigerian Potential Hop Substitutes: Comparative Studies in Beer Brewing

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ABSTRACT

Isomerized hop extract and methanolic extracts of *Azadirachta indica*, *Garcinia kola*, *Gongronema latifolium* and *Vernonia amygdalina* were profiled by the application of Gas Chromatography-Mass Spectrometry (GC-MS). The isomerized hop extract and ethanolic extracts of the plant species were used to brew beers. The aim was to study the GC-MS profiles of all the extracts comparatively and to investigate some physicochemical properties of beers brewed with hop extracts and in comparison, with beers brewed with extracts from the four Nigerian plants. The profiling of the metabolites in hop extracts and those of the Nigerian plants was carried out using Gas Chromatography - Mass Spectrometer. The physicochemical properties of the finished beer products were also carried out using standard methods. Beers brewed with extracts from the Nigerian plants were statistically ranked by the application of Analysis of Variance (ANOVA) to ascertain their potentiality. The GCMS results showed that these plants contained metabolites comparable to those of hops, 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 hops were absent in the Nigerian plants. Isomerized hop, hop leaf, *G. kola* and *V. amygdalina* extracts contained 14, 11, 12 and 9 metabolites respectively while those of *A. indca* and *G. latifolium* contained 10 metabolites each. The physicochemical properties of the brewed beers revealed that the alcohol content in all the beer samples ranged from 3.43-3.75%, total acidity (0.132-0.324%), pH (5.47-5.68), turbidity (5-125NTU), total solids (3.66-8.16%) and bitterness level (25.38-39.62IBU). The concentration of arsenic in the beer samples ranged from 1.44-1.77ppm, cadmium (0.00-0.97ppm) and copper (0.10-2.70ppm). Test of significant (p-value) in all the tested plants was greater than 0.05 at 95% confidence interval. Consequently, the extracts from tested Nigerian plants could be used as suitable substitutes for hops in beer brewing without alteration of physicochemical properties of beer, G. kola having the greatest potential as substitute for hops.

Keywords: *GC/MS, Hops, Nigerian potential hop substitutes, Beer, Brewing, Physicochemical properties*

INTRODUCTION

Beer, a brewed beverage is made principally from malt (partially germinated barley), hop, water and yeast. Beer production worldwide is a viable industry. Among commercial beverages in 2006, beer ranks fourth in per capita consumption behind carbonated soft drinks, bottled water and coffee followed by milk and fruit drinks in the United States of America. Per capita beer consumption rose rapidly during the second world-war, declined during the 1950s and early 1960s, increased before peaking in the early 1980s and has generally leveled-off thereafter [1].

A similar trend is reported of the beer industry in Nigeria by Badmus [2]. He reported that 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. This can be attributed to the country's favorable demographics with populous and vibrant youth and growing middle class. This, along with a growing, largely youth population with increased disposable incomes is the constant drive that increased beer consumption in Nigeria.

Even as Western beer consumption slows down due to the global economic downturn, Nigeria's beer industry continues to thrive. The country has the second largest beer market in Africa, after

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South Africa and with the largest population in Africa, a growing middle class and a large number of drinking beer age, the brewing multinationals are struggling for a position in a market that shows plenty of room for expansion. Indeed, beer drinking has been steadily increasing in recent decades even in countries where alcoholic beverages are not traditional. Hence, beer has become an international drink, especially among young people [3].

Despite the fact that Islamic Sharia law bans the sale and consumption of alcohol in some of Nigeria's Northern states, consumers continue to find means of buying beer. Alcohol in the Northern States is sold in military facilities, which are federal territory and thus not subject to state laws [2].

Beer production in Nigeria has increased recently due to ready markets. This assertion is expressed by the annual consumption rate of beer in Nigeria as shown in Table 1. Hence, the importation of hops to meet the demand of the brewing industries continues to constitute a significant proportion of the Nigerian economy.

Table 1: Annual consumption rate of beer in Nigeria from 2008 to 2011

	Quantity Consumed (mn hl)*
2008	115
2009	126
2010	151.2
2011	151.5

(Source: NIBREWNEWS, 2013) [4]. *million hectoliter

Hops, a minor ingredient in beer, are used for their bittering, flavouring and aroma-enhancing powers. Hops also have pronounced bacteriostatic activity that inhibits the growth of gram-positive bacteria in the finished beer and, when in high concentrations, aids in the precipitation of proteins [5]. The hop plant is grown in the temperate regions of the world, solely to meet the demand of the brewing industry [6]

Azadirachta indica is used in some parts of Nigeria for treatment of malaria, *Garcinia kola* is used in some areas for the treatment of stomachache and gastritis. *Vernonia amygdalina* and *Gongronema latifolium* are widely consumed as vegetables. One thing common to all the four plants is that they are bitter, like hops, but thrive in tropical regions, unlike hops [7].

This piece of work was designed to investigate the profiles of imported hop extracts by the application of gas chromatography-mass spectrometry and contrast to those from the Nigerian plants. It was also designed to find the possibility of these plants serving as substitutes for hops in beer production. The economic importance of such substitution is considerable and forms a reasonable boost for research in this area.

When such substitution is established, the brewing industries in Nigeria will no longer depend on imported hops. This level of raw material freedom will confer definite economic advantages to the Nigerian brewing industry.

MATERIALS AND METHODS

Extraction

Extraction was carried out as explained in our previous works [8, 9]. Except isomerized hop extract that was purchased from Ritchies, England, United Kingdom, four fresh Nigerian bitter vegetables (*G. kola*, *A. indica*, *V. amygdalina* and *G. latifolium*) were procured, sorted and washed in tap water. They were air-dried for10 min., after which they were transferred into an air drought oven, at a temperature of 57 °C for 24 h. The vegetables were allowed to cool at room temperature and were subsequently milled to powder. Five grams (5 g) of each of the vegetable was poured into four different beakers and 20 ml of methanol was added to each of the samples. The samples were transferred to a mechanical shaker which shook the mixtures vigorously and continuously until the mixture formed two layers. The extract was filtered, autoclaved and cooled at a temperature of 20 °C.**Table 1:** Baseline characteristics of patients (intention-to-treat population).

GC-MS technique

GCMS analysis was performed using a Shimadzu GCMS-QP2010 plus (Schimadzu Oceania, Japan) as described by Shellie et al., 2009 [10] and previously adapted in our works [11, 12]. 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 104.1 KPa to provide an initial flow rate of 2ml/min. A 1µl split injection (230oC, 1.5min) was used. The GC temperature gradient was 85oC to 330oC at the rate of 4oC/min and held at 330oC for 10 minutes. Full-scan mass spectra were collected from 85 to 550 mass/charge ratio at a data acquisition rate of 10 spectra/second. The MS transfer line was held at 250oC and the ion source temperature was 200oC. This method was detailed as footnote in each chromatogram.

GC/MS profiling

GC-TOFMS is a benchmark approach for metabolomics data acquisition from chromatographic peaks [13]. The GC component provides excellent sensitivity and sufficiently high data density to permit the deconvolution of overlapping constituent 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 [14]. In the present investigation, a scanning mass spectrometer was used to obtain chromatograms for the samples (Figures 1-6). Spectrum matching is achieved by programming the software to compare the chromatogram of the mass spectra to simulated library peaks [15]. This process had been described previously [11].

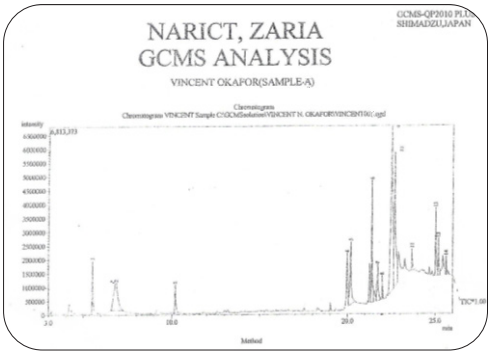


Figure 1: Chromatogram of isomerized hop extract (Sample A)

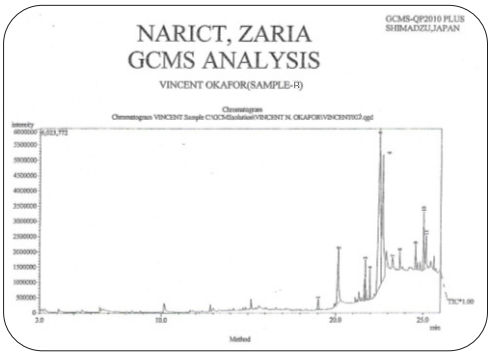


Figure 2: Chromatogram of hop leaf extract (Sample B)

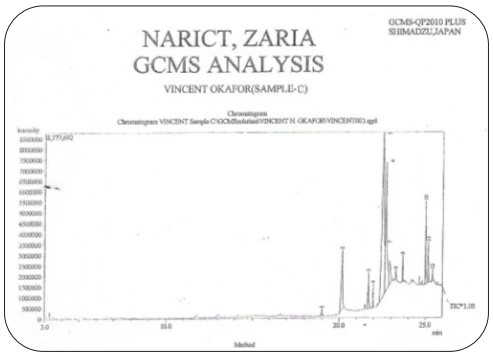


Figure 3: Chromatogram of *G. kola* extract (Sample C)

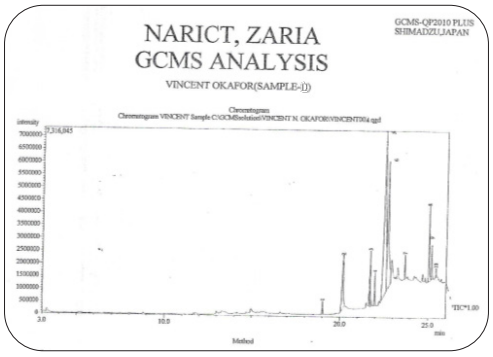


Figure 4: Chromatogram of *A. indica* extract (Sample D)

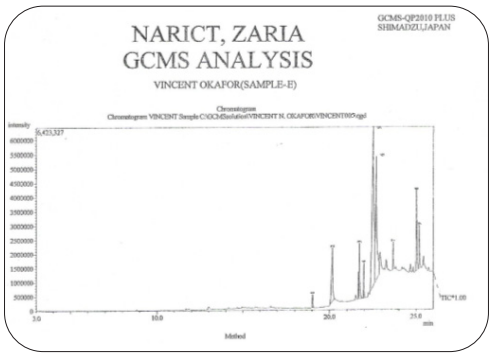


Figure 5: Chromatogram of *V. amygdalina* extract (Sample E)

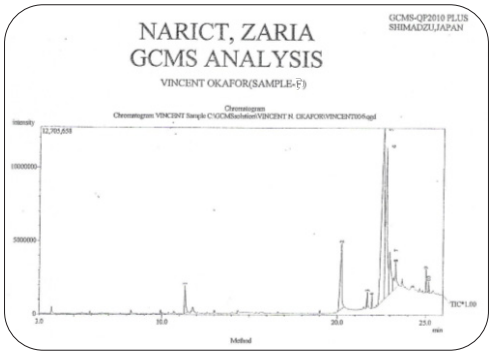


Figure 6: Chromatogram of *G. latifolium* extract (Sample F)

Brewing of beers and determination of physicochemical properties

The beers were brewed at Nigerian Breweries PLC, Ama, Enugu State, Nigeria. The method used in brewing the beers had been described in some literatures [9, 16]. Alcohol content of the samples was determined by distillation method as described by Ceirwyn [17]. Bitterness was determined according to ASBC Beer 23A method [18]. pH was measured by Electrometric method using laboratory pH meter as described by Food Compliance Laboratory Unit of National Agency for Food and Drug Administration and Control (NAFDAC SOP Code: FC: 06.5) [19]. A modified method used by Ceirwyn [17] was used in the determination of total acidity. Turbidity was determined as described by AOAC method 970.14 [20]. The method employed in determining total solids was as detailed by NAFDAC [21]. The concentration of arsenic, cadmium and copper in the samples was analyzed by the method reported by Ubuo, 2009 [22].

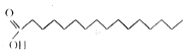
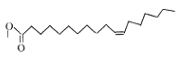
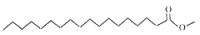
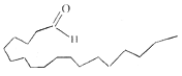
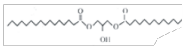
The primary endpoint was assessed in 19 of the 23 patients who completed the study (AZA, 9 patients; ADA, 10) (Figure 1) at weeks 78. In the ITT population, mucosal healing were achieved in 5/16 patients in the AZA group and in 7/12 patients in the ADA group (31.2% and 58.2%, respectively; p=0.24). The PP population revealed endoscopic remission in 3/9 patients in the AZA group and 7/10 patients in the ADA group (33.3% and 70.0%, respectively; p=0.17) (Figure 2).

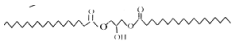
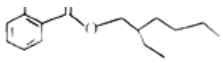
Statistical analysis

Simple statistics (ranking) was employed to determine the significant difference between the controls (hops) and the Nigerian plants. In ranking, we determined the existence of the significant difference among isomerized hop (control), *G. kola, A. indica, V. amyadalina* and *G. Latifolium* on one hand and hop leaf (control), *G. kola, A. indica, V. amygdalina* and *G. latifolium* on the other hand. We employed test of significant difference using ANOVA.

Ranking

Table 2: Metabolites of isomerized hop extract

S/N	Metabolite	Formula	Structure
1.	4,4-Dimethyl-2-buten-4-olide	$C_6H_8O_2$	
2.	1,2-Dimethyl eyelopropane carboxylic acid	$C_6H_{10}O_2$	
3.	2,5-Dimethyl-1-hexanol	$C_8H_{18}O$	
4.	Dehydro-cohumuline acid	$C_{14}H_{18}O_3$	
5.	n-Hexadecanoic acid	$C_{16}H_{12}O_2$	
6.	4,4,5,5-Tetramethyl-bicyclohexyl-6-ene-2,3	$C_{16}H_{24}O_2$	
7.	11-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	
8.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	
9.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	
10.	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	
11.	Hexadecanoic acid, hydroxy-1-3-propanediyl ester	$C_{35}H_{68}O_5$	

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

In the test of significant difference, One Way Analysis of Variance is the most suitable tool as it has the capacity to show the existence of difference at 5% level of significance [23]. In ANOVA, two hypotheses, H0 and H1 are stated and tested for:

H0; there is no significant difference among samples of interest.

H1; there is significant difference among samples of interest.

The result of the p- value (significance value) is used to accept or reject either of the hypotheses.

RESULTS AND DISCUSSION

GC/MS profiles of the extracts

The results presented in Tables 2-7 (metabolite name, formula and structure) extracted from Figures 1-6, GC-MS fingerprints (chromatograms of the extracts) show the profiles of isomerized hop, hop leaf, *G. kola*, *A. indika*, *V. amygdalina* and *G. latifolium* extracts respectively. Isomerized hop, hop leaf, G. kola and V. amygdalina extracts contained fourteen, eleven, twelve and nine metabolites respectively while those of A. indca and *G. latifolium* each contained 10 metabolites.

Table 3: Metabolites of hop leaf extract

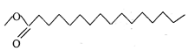
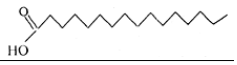
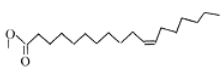
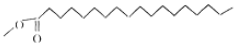
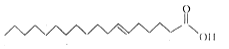
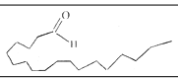
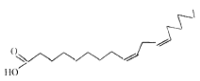
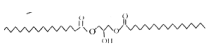
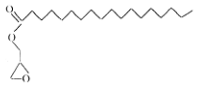
S/N	Metabolite	Formula	Structure
1.	Hexadecanoic, methyl ester	$C_{17}H_{34}O_2$	
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	
6.	Octadecanoic acid	$C_{18}H_{36}O_2$	
7.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$	
8.	Octadecanoic acid, 2-hydroxy-1, 3-propandiyl ester	$C_{39}H_{76}O_5$	
9.	Lupulone (beta-lupulic acid)	$C_{26}H_{38}O_4$	
10.	9-Hexadecenal	$C_{16}H_{30}O$	
11.	Octadecanoic acid, oxiranyl methyl ester	$C_{21}H_{40}O_3$	

Table 4: Metabolites of G. kola extract

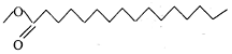
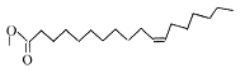
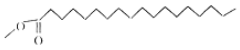
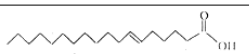
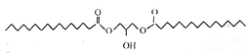
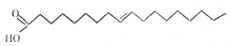
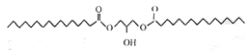
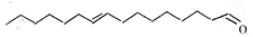
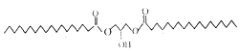
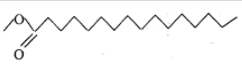
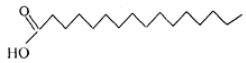
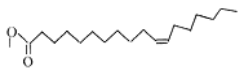
S/N	Metabolite	Formula	Structure
1.	Hexadecanoic, methyl ester	$C_{17}H_{34}O_2$	
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	
3.	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	
6.	Octadecanoic acid	$C_{18}H_{36}O_2$	
7.	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$	
8.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$	
9.	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$	
10.	9-Hexadecenal	$C_{16}H_{30}O$	
11	Octadecanoic acid, 2-hydroxyl-1, 3-propanediyl ester	$C_{39}H_{76}O_5$	
12	Hexadecanoic acid, 2, 3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	

Table 5: Metabolites of A. indica extract

S/N	Metabolite	Formula	Structure
1.	Hexadecanoic, methyl ester	$C_{17}H_{34}O_2$	
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	

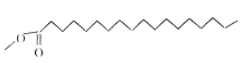
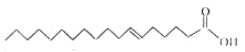
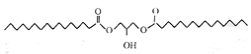
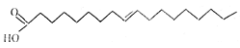
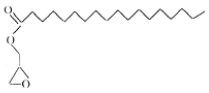
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	
6.	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	
7.	Hexadeca-noic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$	
8.	9,12-Octadeca-dienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$	
9.	Octadecanoic acid, oxiranyl-methyl ester	$C_{21}H_{40}O_3$	
10.	Hexadecanoic acid, 2, 3-di-hydroxypropyl ester	$C_{19}H_{38}O_4$	

Table 6: Metabolites of V. amygdalina extract

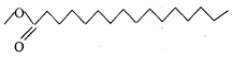
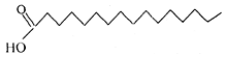
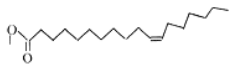
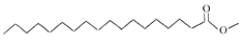
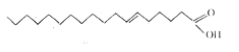
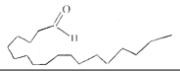
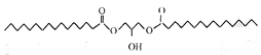
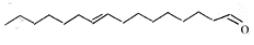
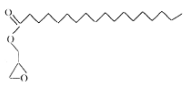
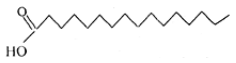
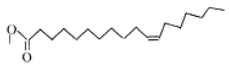
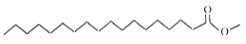
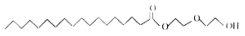
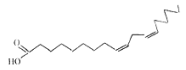
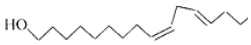
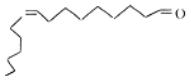
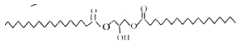
S/N	Metabolite	Formula	Structure
1.	Hexadecanoic, methyl ester	$C_{17}H_{34}O_2$	
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	
3.	11-Octadeceno-ic acid, methyl ester	$C_{19}H_{36}O_2$	
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	
6.	Octadecanoic acid	$C_{18}H_{36}O_2$	
7.	Hexadeca-noic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$	
8.	9-Hexadecenal	$C_{16}H_{30}O$	
9.	Octadecanoic acid, oxiranyl methyl ester	$C_{21}H_{40}O_3$	

Table 7: Metabolites of G. latifolium extract

S/N	Metabolite	Formula	Structure
1.	Benzoic acid, 2-(aminocar-bonyl)	$C_8H_7NO_3$	
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	
3.	11-Octadeceno-ic acid, methyl ester	$C_{19}H_{36}O_2$	
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	
6.	Octadecanoic acid 2-(2-hy-droxyl-ethoxy) ethyl ester (Aquacera)	$C_{22}H_{44}O_2$	
7.	9,12-Octadeca-dienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$	
8.	9,12-Octadeca-dienoic-l-ol	$C_{18}H_{34}O$	
9.	Octadecanoic acid, oxiranyl-methyl ester	$C_{16}H_{30}O$	
10.	Octadeca-noic acid, 2-hydroxy-1, 3-propandiyl ester	$C_{19}H_{38}O_4$	

From Table 8, it is evident that 4,4-dimethyl-2-buten-4-olide (C6H8O2); 1,2-dimethyl cyclopropane carboxylic acid (C6H10O2); 2,5-dimethyl-2-hexanol (C8H18O); 4,4,5,5-tetramethyl bicyclo hexyl-6-ene-2,3,-dione (C16H24O2); 1,2-benzen dicarboxylic acid bis (2-ethyl hexyl) ester (C24H38O4) and dehydro-cohumulunic acid (C14H18O3) are present in isomerized hop extract only. It is also observed that hop leaf extract only contained lupulone (C26H30O4), a -acid known as beta-lupulic acid and octadecanoic acid oxiranyl methylester (C21H46O3). All the extracts contained hexadecanoic acid (C16H32O2), octadecenoic acid methyl ester (C19H36O2), octadecanoic acid methyl ester (C19H38O2) and 6-octadecenoic acid (C18H34O2). The extracts of hop leaf, isomerized hop, G. kola, A. indica and V. amygdalina contained octadecanoic acid (C18H36O2) in common while the extracts of isomerized hop, hop leaf, G. kola, A. indica and G. latifolium contained 9, 12-octadecadienoic acid, the grape seed oil (C18H32O2) in common. The extracts of isomerized hop, hop leaf, G. kola, and G. latifolium only contained octadecanoic acid, 2-hydroxyl-1, 3-propandiyl ester (C39H76O5). Another significant observation is that each of the extracts of hop leaf, G. kola, V. amygdalina and G. latifolium contained 9-hexadecenal (C16H30O) which was absent in extracts of isomerized hop and A. indica. Also, each of the extracts of hop leaf, G. kola, A. indica and V. amygdalina contained hexadecanoic acid methyl ester (C17H34O2). Hexadecanoic acid methyl ester was not present in both the extracts of isomerized hop and G. latifolium.

Table 8: Abundance of Metabolites in the Extracts

S/N	Metabolite	Isomerized hop	Hop leaf	G. kola	A. indica	V. amygdalina	G. latifolium
		Abundance					
1.	4,4-dimethyl-2-buten-4-olide	0.362	Nf	Nf	Nf	Nf	Nf
2.	1,2-dimethyl-cyclopropane carboxylic acid	0.990	Nf	Nf	Nf	Nf	Nf
3.	2,5-dimethyl-2-hexanol	0.268	Nf	Nf	Nf	Nf	Nf
4.	Dehydro-cohumulunic acid	0.533	Nf	Nf	Nf	Nf	Nf
5.	4,4,5,5,tetramethyl-bicyclo hexyl-6-ene-2,3-dione	0.925	Nf	Nf	Nf	Nf	Nf
6.	1,2-benzenedicarboxylic, bis (-2-ethylhexyl) ester	0.114	Nf	Nf	Nf	Nf	Nf
7.	Hexadecanoic acid	0.784	0.954	0.930	0.957	0.923	1.069
8.	Octadecenoic acid, methyl ester	0.369	0.314	0.284	0.434	0.512	0.077
9.	Octadecanoic acid, methyl ester	0.121	0.245	0.162	0.236	0.263	0.065
10.	6-octadecenoic acid	2.896	4.355	4.409	4.496	4.342	4.461
11.	Octadecanoic acid	1.792	2.556	2.331	2.458	2.406	Nf
12.	Hexadecanoic acid, 2-hydroxy -1,3-propanediyl ester	0.124	Nf	0.192	0.195	0.225	Nf
13.	9,12-octadecadienoic acid (grape seed oil)	0.465	0.126	0.104	0.715	Nf	0.795
14.	Octadecanoic acid, 2-hydroxyl -1,3-propanediyl ester	0.257	0.163	0.284	Nf	Nf	0.061
15.	Hexadecanoic acid, methyl ester	Nf	0.113	0.069	0.141	0.146	Nf
16.	Lupulon (beta-lupulic acid)	Nf	0.202	Nf	Nf	Nf	Nf
17.	Octadecanoic acid, oxiranyl methyl ester	Nf	0.413	Nf	0.283	0.351	Nf
18.	9-hexadecenal	Nf	0.559	0.707	Nf	0.831	0.146
19.	2-methyl-3,13-octadecadienol	Nf	Nf	0.423	Nf	Nf	Nf

However, there are metabolites which were present in the local substitutes that were conspicuously absent in imported hops even though the Nigerian plants contained these metabolites differently, e.g. while G. kola alone contained 2-methyl-3, 13-octadecadien-1-ol ($C_{19}H_{36}O$), G. latifolium alone contained octadecanoic acid -2-(2-hydroxyethoxy) ethylester (C22H44O2) which is aqua cera and 9, 12-octadecadiene-1-ol (C18H34O). All these constituents were completely absent in imported hops. This minor differences and major similarities in the constitution of chemical constituents in the local plants and those of imported hops is in agreement with the observation of Shellie et al. (2009) [9] in their varietal characterization of hop by GC-MS 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) [24] were more pronounced while their chemical properties did not differ much.

Physicochemical properties

Table 9 shows the result of physicochemical properties of all the beer samples. Alcohol content ranged from 3.43 to 3.75 (% v/v) with samples A and D having the highest alcohol content. The results from the study by Ifeanyi and Ihenatuoha [25] showed an agreement with the present work; both having similar alcoholic contents which might be as a result of low rate of fermentation of the beer samples.

Table 9: Physicochemical properties of the beer samples

Sample	A	B	C	D	E	F
Parameter						
Alcohol (% v/v)	3.75	3.43	3.43	3.75	3.43	3.43
Bitterness (IBU)	39.62	30.91	25.38	33.87	29.12	27.56
pH	5.57	5.68	5.57	5.47	5.50	5.49
Total acid (% v/v)	0.228	0.114	0.132	0.324	0.288	0.288
Turbidity (NTU)	50	100	110	125	5	6
Total solid (%)	6.34	3.56	3.86	8.16	4.72	5.85
Arsenic (ppm)	1.50	1.44	1.77	1.52	1.62	1.60
Cadmium (ppm)	0.00	0.81	0.97	0.96	0.00	0.68
Copper (ppm)	2.70	1.99	2.39	0.10	1.32	1.46

Source: Okafor et al., 2016 [12]

The bitterness level in the samples and the control ranged between 25.38 and 39.62IBU. The pH of the beers shows that sample B has the highest pH value of 5.57 while B has the lowest pH of 5.47 These results agree with that of Omuku *et al.*, 2012 [26]. pH is an important factor in brewing quality beer. The pH levels during various stages of the brewing process affect extract potential, beer colour, hot-break formation, foam stability, hop oil extraction, hop bitterness and lauterability of the beer [27]. It is also an important consideration for beer quality during storage as a low pH inhibits bacterial growth. pH affects almost all the physical, chemical and biochemical reactions that occur within the brewing process. Brewers who understand the factors that affect pH and how to manage them during the brewing process will be able to consistently produce good beers. Although pH is clearly an important variable in the brewing process, it rarely requires a great deal of attention from the brewer [28]. It is evident from Table 8 that the total acidity of all the beer samples are within the same range except samples B and C. These results are in agreement with that of Okafor *et al.*, 2017 [11] and other works [16, 29, 30, 31]. Interestingly, all the beer samples exceeded the 0.1% minimum allowed total acidity of NAFDAC's recommendation in lager beers in Nigeria (NAFDAC, 2013) [32]. The turbidity of all the samples as presented in Table 8 ranged between 125 and 5 NTU. Sample E was virtually the clearest and D, the most turbid (cloudy). Samples B, C, and D were 100, 110, and 125 respectively, all in Nephelometric Turbidity Unit (NTU) and were especially high compared with the turbidity in samples E (5 NTU) and F (6 NTU). The turbidity in sample A was 50 NTU. These results are not in agreement with the turbidity standards (0.15 NTU) for drinking water in the United State (USEPA, 2009) [33]. Furthermore, the turbidity in samples B, C and D are comparable to one another; samples E and F are also comparable to each other while sample A is not comparable to any of the samples. These discrepancies that exist in turbidity values of the beer samples could easily be explained by the fact that length of time each beer sample was exposed to the atmosphere during hopping was not constant. During each period, fugitive harmful organisms such as bacteria, viruses, protozoa, moulds, and wild yeasts could infect the beer. The more the beer is exposed to the atmosphere, the more the loads of these organisms and of course the more the beer develops a biological haze and goes turbid. These results could explain the reason why excess consumers of beer often complain about gastrointestinal diseases because in drinking water, the higher the turbidity level, the higher the risk that people may develop gastrointestinal diseases (Mann *et al.*, 2007) [34]. From the Table above, the percentage total solids ranged between 3.86 and 8.16 with sample D having the highest percentage of 8.16 and the lowest, sample B, with a value of 3.86%. The percentage total solids of the beer in samples B, C, and E are within the permissible maximum limits of total solids in beer. The National Agency for Food and Drug Administration and Control (NAFDAC)'s permissible maximum limit of total solids in beer is 5% [35]. The percentage total solids in samples A and D are above the permissible limit while that in F is slightly above the limit. From the result presented in Table 8, arsenic concentration in the samples ranged between 1.44 – 1.77mg/L, with sample C having the highest concentration of 1.77mg/L, and sample B, the lowest concentration of 1.44mg/L. The FAO/WHO maximum permissible limit of arsenic in drinking water is 10µg/L (FAO/WHO, 2011) [36]. The arsenic content of the beer samples was above the maximum permissible limit of arsenic in drinking water. In Britain, the level of arsenic in lagers may not exceed 0.2mg/kg [6]. Again, the concentration of arsenic in the beer samples investigated is much more above this level. The explanation for this may be the region of growth of the raw materials used in the production. cadmium concentration in the beer samples ranged between 0.97ppm and 0.00pm with sample C having the highest concentration of 0.97 mg/L and not detected in samples A and E. The concentrations of cadmium in samples B, D and F are 0.81ppm, 0.97ppm and 0.68ppm respectively. These results differ significantly with the result of Ubuoh (2013) [22] except in samples A and E. The World Health Organization (WHO) [37] in 2001 reported a cadmium content varying from 12.90 – 14.30µg/l in Brazilian beers. Also, the Standard Organization of Nigeria, SON (2003) [38] gave the limit for Cd content in drinking water as Iµg/kg bw/day. All the beer samples examined had Cd concentrations above that in Brazilian beers and the permissible limit in drinking water with the exception of beer samples A and E where Cd was not detected. Copper content of the beer samples

varied between 2.70ppm and 0.10ppm, with sample A having the highest concentration of 2.70ppm, and the lowest being sample D with 0.10ppm. The permissible limit for copper in drinking water in Nigeria is 1.0ppm according to some regulatory bodies such as (Standard Organization of Nigeria (SON) [38], World Health Organization (WHO) [39]. The British Food Standard Committee has recommended limits of 7.0ppm and 5.0ppm for copper and zinc respectively in wines and beers [6]. The Copper content of the beer samples analyzed was above the permissible limit for drinking water in Nigeria except in Sample D but below the limit in British beers. It is well noticed that the concentrations of the metals in the finished beer samples differ among themselves because metals in beer are derived from various raw materials, equipment and brewing processes [40].

RANKING OF PHYSICOCHEMICAL PROPERTIES OF BREWED BEERS

Isomerized hop

The p-value of the test as shown in Table 10 is 0.705 which is greater than 0.05. We then have enough evidence to accept the null hypothesis and conclude that there is no significant difference among the samples studied.

Table 10: PANOVA for comparison of physicochemical properties of beers brewed with Isomerized hop and the Nigerian plants

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	1497.217	4	374.304	0.543	0.705
Within Groups	27565.760	40	689.144		
Total	29062.978	44			

The output of the post hoc test (Table 11) shows that G. *kola* has the highest significance value of 0.696 which implies that the plant is the closest among all the samples to isomerized hop. Other Nigerian plants (*A. indica, V. amygdalina and G. latifolium*) have significance values less than 0.696 but greater than 0.05. This implies that extracts from the plants are insignificantly different from isomerized hop extract.

Table 11: Post hoc tests for comparison of isomerized hop extract and those of Nigerian plants

(+A) Factor	(*J) Factors	Mean Difference (A-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	C	-4.86600	12.37510	.696	-29.8770	20.1450
	D	-7.71622	12.37510	.536	-32.7272	17.2948
	E	6.52333	12.37510	.601	-18.4877	31.5343
	F	6.37222	12.37510	.609	-18.6388	31.3832

+A = Izomerized hop extract
*J = Nigerian plants

Hop leaf

From Table 12, it is seen that the p-value of the test is 0.743 which is greater than 0.05. We therefore have enough evidence to accept the null hypothesis and conclude that there is no significant difference among the plants considered.

Table 12: ANOVA for comparison of physicochemical properties of beers brewed with Hop leaf and the Nigerian plants.

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	1632.103	4	408.026	0.490	0.743
Within Groups	33324.703	40	833.118		
Total	34956.806	44			

It is seen from the post hoc test (Table 13) that in the comparison of hop leaf with the Nigerian plants, G. kola has the highest significance value of 0.964 which implies that G. *kola* is the closest among the plants to hop leaf (control). Other Nigerian plants (*A. indica, V. amygalina and G. latifolium*) have significant values less than 0.743 which are higher than 0.05. This means that extracts from the plants are not significantly different from that of hop leaf.

Table 13: Post hoc tests for comparison of hop leaf extract and those of Nigerian plants

(+B) Factor	(*J) Factors	Mean Difference (B-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
B	C	-.61533	13.60651	.964	-28.1151	26.8845
	D	-3.46556	13.60651	.800	-30.9653	24.0342
	E	10.77400	13.60651	.433	-16.7258	38.2738
	F	10.62289	13.60651	.400	-16.8769	38.1227

+B = Izomerized hop extract
*J = Nigerian plants

CONCLUSIONS AND RECOMMENDATION

This study has shown that the extracts from tested Nigerian plants could be used as suitable substitutes for hops in beer brewing without alteration of the physicochemical properties of beer irrespective of the fact that the profiles of the chemical metabolites in imported hops and the Nigerian plants differed significantly. Consequently, academic activity in the area of mixtures/blends of extract of plant species which mimic hop taste is strongly recommended.

COMPETIG INTERESTS

Authors declare that they have no competing interests.

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AUTHOR'S CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author VNO designed and wrote the entire manuscript and sourced most of the data and literature and as well supervised authors UWO and RIA during laboratory work. Author UWO also provided some literature on GC-MS while author RIA in addition assisted in the procurement of research materials and literature on beer. All authors read and approved the final manuscript.

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