
Vincent Nwalieji Okafor¹, Ifeyinwa Blessing Tabugbo¹, Regina Igwe Anyalebechi², Ugochukwu Wilson Okafor³ and Joy Ngozika Obiefuna¹

¹Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Nigeria.
²Department of Science Laboratory Technology, Federal Polytechnic, Oko, Anambra State, Nigeria.
³National Board for Technology Incubation, Federal Ministry of Science and Technology, Abuja, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. Author VNO designed and wrote the entire manuscript and sourced most of the data and literature. Author IBT provided some literature on PAHs, author RIA assisted in providing literature on beer and author UWO provided literature on GC-MS and GC-FID while author JNO assisted in interpreting some of the data. All authors read and approved the final manuscript.

ABSTRACT

The Nigerian economy depended mainly on crude oil during the era of oil boom of 1973 which lasted up till 1983. Agriculture was grossly neglected by successive governments. Following the economic recession that occurred years after and due to fall in crude oil price, the Nigerian government began to advocate for economic diversification. Consequently, agriculture became the area of interest and priority for industrial raw material sources. Unfortunately, Nigeria had imbibed the tradition of importing raw materials for all her industrial productions thereby creating unfavourable balance of trade between Nigeria and her foreign trading partners thus resulting in increase in the prices of finished products. Beer production is not exempted from the price increase...

*Corresponding author: E-mail: vnw.okafor@unizik.edu.ng;*
since its raw materials are equally imported with their attendant problems on Nigeria's foreign exchange. One of such raw materials is hops. The hop (\textit{Humulus lupulus} L.) is a perennial dioecious climbing plant of hemp (cannabis) family and belonging to the order (urticales) which are grown in the temperate regions of the world, solely to meet the demand of the brewing industry. Hop extracts give beer its bitter taste, improve foam stability and act as antiseptics towards microorganisms. The quest to substitute hops with some tropical bitter vegetables in Nigeria's brewing industry dates back to 1983 and since that time, many have compared hop extracts with those of Nigerian bitter plants such as Garcinia kola, Azadirachta indica, Vernonia amygdalina and Gongronema latifolium. This review takes a critical look on the efforts made so far since 1983 in investigating the potentiality of using Nigerian bitter plant extracts as suitable substitute for those of hop in the Nigerian brewing industry with special emphasis on Gas Chromatography Mass–Spectrometry (GC–MS) and Gas Chromatography–Flame Ionization Detector (GC–FID) techniques. It was concluded that none of the Nigerian plants has perfect potential as suitable substitute for hops in the Nigerian brewing industry. Consequently, further research efforts in the area of mixtures/blends of extract of plant species which mimic hop taste is strongly recommended.

Keywords: Hops; hop substitutes; beer brewing; GC–MS; GC–FID.

1. INTRODUCTION

1.1 Background

The Webster's Dictionary [1] defined beer as an alcoholic drink made from yeast fermented malt, flavoured with hops. The word beer is derived from the latin word \textit{bibere} which means to drink [2]. From medieval times, herbs have been used to flavour and preserve fermented malt liquors but only hop inflorescence is used on a commercial scale today [3]. In Nigeria, hops are imported, and with the expansion of the brewing industry huge amounts of foreign exchange are being spent by this sector for importation of hops.

As far as brewing industry is concerned, hops are the dried cone of the female hop plant and products made from them. The hop cone or strobilus, the female inflorescence consists of a valueless stipular bracts and seed bearing bracteoles attached to a central axis or strig. At the base of the bracteoles the lupulin glands and seeds develop as the hop resins [4]. The brewing value of the hop is found in its resins and essential oils. Peacock (2009) [5] put it that the brewing value of the hop is found in hop resins and essential oils that are contained in the lupulin glands of the female hop cone. These contain bitter resins and ethereal oils which supply bittering and aroma components of beer. Hop resins are the most valuable and most characteristics components of hops. They give beer its bitter taste, improve foam stability and act as antiseptics towards microorganisms [6]. In the traditional brewing process, hops are boiled with wort in a copper vessel for 1-2 hours, during which the resins go into solution and are isomerized to produce the bitter principles of beer. The majority of essential oil constituents will be lost during 2 hours of boiling. So to increase the hop aroma of their beers brewers either add a portion of choice aroma hops late in the boil or add them to the beer during conditioning  – a process known as dry hopping [7].

Hop resins are sub-divided into hard and soft based on their solubility. Hard resins are of little significance as they contribute nothing to the brewing value, white soft resins contribute to the flavouring and preservative properties of beer. Alpha and beta acids are two compounds present in the soft resins and are responsible for bitterness. Alpha acids are the precursors of beer bitterness since they are converted into iso alpha acids in the brew kettle. They are therefore responsible for about 90% of the bitterness in beer [8].

The three major components of alpha acids are humulone, cohumulone and adhumulone. The beta acids include lupulone, colupulone, and adlupulone which are only marginally bitter [3].

The structures of alpha acids and beta acids, compounds (1) and (2) are shown in Figs. 1 and 2 respectively.

In Table 1, when \( R = \text{CH}_3\text{CH(\text{CH}_3)}_2 \), the alpha-acid in Fig. 1 is humulone. When \( R = \text{CH(\text{CH}_3)}_2 \), the alpha-acid is cohumulone and when \( R = \text{CH(\text{CH}_3)}_3\text{CH}_2\text{CH}_3 \), the alpha-acid is adhumulone.

Similarly, in Table 2, when \( R = \text{CH}_2\text{CH(\text{CH}_3)}_2 \), the \( \beta \)-acid in Fig. 2 becomes lupulone. When \( R = \text{CH(\text{CH}_3)}_2 \), the \( \beta \)-acid in Fig. 2 becomes humulone.
CH(CH₃)₂, it represents colupulone and when R is CH(CH₃)CH₂CH₃, the β-acid is adlupulone. When hops are added to the boiling wort in the kettle, their alpha acid (1) go through isomerization and are converted to iso-alpha acids, (3) (Fig. 3) [5]. Alpha acids are isomerized into iso alpha acids using dilute alkaline solutions and this isomerization is catalyzed by calcium or magnesium ions either in methanol or the solid state, without the formation of humulinic acid [3, 8]. In this conversion, humulone is converted to iso-humulone, cohumulone to iso-cohumulone and adhumulone to iso-adhumulonme.

![Fig. 1. Structure of alpha acids](image1)

Fig. 1. Structure of alpha acids

Although, hops that have high alpha acid contents are preferred for their bittering and flavouring properties, hops are also selected based on the character of their oils. Oils are largely responsible for the characteristic aroma of hops and either directly or indirectly, for the overall perception of hop flavour. Hops selected on the basis of their oil content are often referred to as aroma or “noble” type hops. Oils also tend to make beer’s bitterness a little more pronounced and enhance the body or mouth feel of the beer [3].

![Fig. 2. Structure of beta acids](image2)

Fig. 2. Structure of beta acids

Beer brewery activity involves the solubilization of the carbohydrate content of grains by malting, grinding and boiling to extract sugars. This process also extracts substances such as fats from the grains and may require a degree of de-fatting before actual extraction is established. The process of conversion to alcohol beer is done using special cultured yeast colonies such as *Saccharomyces cerevisiae* for top fermenting and *Saccharomyces carisbergensis* or *Saccharomyces uvarum* for bottom fermenting. Essential change here is conversion of glucose to ethanol. The addition of processed hop inflorescence allows some foam stability extracted from the hop product as small polymeric units. Furthermore, flavours as phytochemicals from the hop additives are in classes such as flavones and terpenoids. Another class, the alkaloids are the source of the bitter tastes. It is therefore possible to supply these qualities, which distinguishes beers from plant equivalent of hops or hop produce. This substitution of chemical moieties from plants equivalent of hops with emphasis on GC–MS and GC–FID techniques is the bases of this review.

### Table 1. Alpha acids and % alpha acids before isomerization

<table>
<thead>
<tr>
<th>Alpha-Acid</th>
<th>R=</th>
<th>% of Alpha-acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humulone, C₂₁H₃₀O₅</td>
<td>Iso-butyl CH₂CH(CH₃)₂</td>
<td>40 – 80</td>
</tr>
<tr>
<td>Cohumulone, C₂₀H₂₈O₅</td>
<td>Iso-propyl CH(CH₃)₂</td>
<td>17 – 50</td>
</tr>
<tr>
<td>Adhumulone, C₂₁H₃₀O₅</td>
<td>Sec-butyl CH(CH₃)CH₂CH₃</td>
<td>5 – 15</td>
</tr>
</tbody>
</table>

(Source: Peacock, 2009) [5]

### Table 2. Beta acids and % beta acids before isomerization

<table>
<thead>
<tr>
<th>Beta-Acid</th>
<th>R=</th>
<th>% of Beta-acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupulone, C₂₆H₃₈O₄</td>
<td>Iso-butyl CH₂CH(CH₃)₂</td>
<td>15 – 60</td>
</tr>
<tr>
<td>Colupulone, C₂₆H₃₈O₄</td>
<td>Iso-propyl CH(CH₃)₂</td>
<td>35 – 80</td>
</tr>
<tr>
<td>Adlupulone, C₂₆H₃₈O₄</td>
<td>Sec-butyl CH(CH₃)CH₂CH₃</td>
<td>5 – 15</td>
</tr>
</tbody>
</table>

(Source: Peacock, 2009) [5]
Fig. 3. Alpha acids isomerized to Iso alpha acids

1.2 Brief History of Beer

Beer is the world’s oldest and most widely consumed alcoholic beverage, possibly dating back to the early Neolithic or 9,000 Before Christ (BC), and recorded in the written history of ancient Egypt and Mesopotamia [9]. The invention of bread and beer has been argued to be responsible for man’s ability to develop technology and build civilization. Beer was spread in Europe by Germanic and Celtic tribes as far back as 3,000 B.C. and it was mainly brewed on a domestic scale [10].

The U.S. beer industry started in the 1840s and 1850s with the introduction of lager style beers, brought by German immigrants [11]. Before that point, beers were heavily oriented towards ale, porter and stout and were mostly brewed at home. At about the same time, several technological advances occurred that led to the development of the U.S. beer industry. Mechanical refrigeration greatly aided in the production as well as the storage of beer. Pasteurization was also adopted during this period, which opened the way for wide-scale bottling and off-premise consumption of beer. By 1850, there were about 430 breweries in the United States producing about 750,000 barrels of beer annually as commercial brewers began to grow in size and number, and by the late nineteenth century, there were almost 1,300 breweries [12].


2. PROPERTIES OF BEER

2.1 Physicochemical Properties of Beer

Beer is a complex mixture; over 400 different compounds have been characterized in beer which, in addition contains macromolecules such as proteins, nucleic acids, carbohydrates and lipids. Some of the constituents of beer are derived from the raw materials that survive the brewing process unchanged. Others are the result of chemical and biochemical transformation of the raw materials during malting, mashing, boiling, fermentation and conditioning. Together, all these constituents make up the character of beer, but in general, different beers contain different proportions of the same compounds rather than novel constituents. Nevertheless, accidental or deliberate contamination of beer with micro-organism other than yeast may well produce new metabolites [3,14,15].
Beer constituents can be divided into volatile and non-volatile components. The volatile components are responsible for the aroma or bouquet of beer. The non-volatile constituents of beer include inorganic salts, sugars, amino acids, nucleotides, polyphenols, and the hop resins, together with macromolecules such as polysaccharides, proteins, and nucleic acids [3, 14]. The major cations are potassium, sodium, magnesium, and calcium with the anions, chloride, sulphate, nitrate and phosphate. The minor cations include iron, copper, zinc, manganese, lead, arsenic and phosphorous. Fluoride can also be found in trace amount in beers [16].

One way of classifying the organic constituents of beer is with regards to the heteroatoms present. Beer contains only trace amount of hydrocarbons; the majority of the constituents contain carbon, hydrogen, and oxygen. Small amount of nitrogen-containing constituents are present and phosphorous is associated with some of these. Only trace amounts of sulphur-containing compounds are present but some of these are very potent flavouring agents [16].

Methods for beer analysis have been published by, inter alia, the European Brewery Convention-Analytica [17], the American Society of Brewing Chemists [18] and the Institute of Brewing [19]. Not all the methods described refer to the estimation of specific constituents. Some give methods, for other chemical and physical parameters found useful in the quality control of the brewing process.

Some analytical data for beers include percentage alcohol, reducing sugars, pH, bitterness, volume of carbon (IV) oxide (CO₂), volume of sulphur (VI) oxide (SO₃), dissolved oxygen, colour, haze, foam head retention and viscosity [3,20]. The alcohol content of beer is usually regarded as a measurement of its strength. Ethanol, one of the principal products of yeast metabolism is believed to contribute strongly to the body of beer [21].

Carbon dioxide is a natural product of fermentation and beers should contain 3.5 – 4.5/g; the sparkle of a beer when uncapped is due to the evolution of carbon dioxide and beer should obviously contain sufficient carbon dioxide to impart this quality [22]. The colour of beer is largely determined by the melanoids and caramel present in the malt and adjuncts used but further caramelization occurs during wort boiling. Minor adjustments of the colour of beer can be made by the addition of caramel either to the copper or with primings. Viscosity of beer can be a useful figure reflecting the contents and degradation states of various contributory factors, such as β-glucan, derived from the wort [23]. One of the properties of beer appreciated by many consumers is the foam head that develops as the glass is filled. It is generally reckoned desirable that this head should persist and not collapse while the beer is being drunk. Beer is distinguished from all other beverages by the formation of a stable foam head, the physical and chemical properties of which had been reviewed [24]. A good head of dense stable foam on a glass of beer is visually appealing and such beer always has 'mellow' palate [22]. That author used the terms body and palate fullness as synonyms for "mellowness". Among the compounds in beer contributing to the formation of foam and which are also important in beer mouth feel are proteins, polyphenols, glycerol, carbohydrates (dextrins and β-glucan), ethanol and CO₂ [25-27].

Turbidity is the cloudiness or haziness of a fluid caused by large numbers of individual particles that are generally invisible to the unaided eyes, similar to smoke in air. Beers infected with bacteria or wild yeast will rapidly go turbid and develop a biological haze but with the wide spread use of pasteurization and sterile filtration such infections are fairly rare [3]. However, uninfected beers when stored for any length of time, usually in bottle also become cloudy and deposit a haze. Such beers are usually unacceptable and the rate of development of this non-biological haze determines the shelf life of bottled beer [28].

Another important parameter usually underestimated is the pH of beer. pH is the negative logarithm of the effective hydrogen ion concentration or hydrogen activity in gram equivalents per litre used in expressing both acidity and alkalinity on a scale whose values run from 0 to 14 with 7 representing neutrality, numbers less than 7, increasing acidity and numbers greater than 7, increasing alkalinity [29].

2.2 Organoleptic Properties of Beer

The final arbiter of beer quality is the palate of the consumer and this can show wide variations between individuals, between geographical areas, and even from occasion to occasion. Quality is defined as degree of excellence, relative nature, or kind, or character, and
accordingly the brewer refers to many varieties of ale, stout and lager which he brews to satisfy the varied demands as different qualities. When the customer has chosen the quality he wishes to drink, he demands that his beverages shall have the degree 'excellence' which he expects and that this shall not change from day to day. Much of the brewers' art is therefore concerned with quality control, with producing a constant product from variable raw materials by a biological process [3].

The enjoyment of a glass of beer may be received by many senses; the sight may be attracted first by, for example, the clarity of a pale ale or the rich creamy head of a stout. As the glass is raised to the lips, the aroma of the beverage, possibly the bouquet of the essential oils of hops may excite the nostrils [3]. Then, as the liquid flows over the taste buds in the back of the mouth and further volatile products diffuse into the back of the nose, the flavour of the beverage is perceived. Finally, the beer enters the body where the alcohol is rapidly absorbed into the bloodstream and exerts its well-known physiological and psychological effects. Other beer constituents such as the simple sugars will also be rapidly absorbed into the blood stream, but the dextrins will be hydrolyzed before absorption. The alcohol and carbohydrates together are responsible for the nutritive value of beer. In addition, beer is a rich source of the B-group vitamins [3].

Flavour has been described as complex sensation comprising taste, odour, roughness or smoothness, hotness or coolness, pungency or blandness [30]. If we consider beer within this context, taste and odour are undoubtedly the most important properties. Texture refers more to solid foodstuffs than liquids but is probably related to what is referred to as “palate fullness” or ‘body’. This ill-defined beer property is thought to be related to the concentration of macromolecules, principally β-glucans, proteins and melanoids in the beer [3].

The importance of the temperature at which the beer is served is recognized throughout the world, although nations do not agree as to what is the optimum temperature. In general, bottom fermented beers are drunk at lower temperatures (0 -10°C) than those produced by top fermentation (10 – 20°C). With regards to the above definition, beers lack pungency and indeed, many can be regarded as bland. One other property of food and drink akin to flavour, and not mentioned in the above definition is astringency (the production of dryness in the mouth). This property is shown by many compounds, in particular polyphenols such as anthocyanogens, melanoids and the principal amino acids in beer, proline [3].

Taste is defined as the product of the chemical sensory system of the oral cavity. Two types of chemical receptors are recognized: free nerve endings, which occur throughout the oral cavity, and taste buds. The free nerve ending possesses no recognizable receptors and are responsible for the perception of pungency and astringency. Taste buds are neural complexes of 25 – 50 specialized cells which occur in localized areas of the oral cavity. They occur on the back, tip and sides of the tongue [3].

3. EFFORTS IN THE SUBSTITUTION OF HOPS

There has been a growing trend towards sourcing of local substitutes for industrial raw materials in Nigeria and therefore, a lot of efforts have been made since 1983 for the substitution of hops with local raw materials in the brewing industry. The quest for alternative sources of hops in traditional beer brewing is not given spirited efforts in Nigeria only. In USA for example, Schuina et al. [31] recently sought to substitute hops artichoke (Cynara scilymus L) in the production of craft lager beers. They concluded that the use of Cynara scilymus L as a substitute for hops in craft beer brewing is feasible and as such recommended large scale production of beers with Cynara scilymus L.

3.1 Efforts in Physicochemical Properties of Beers

The potentiality of four Nigerian bitter plants namely Garcinia kola, Azadirachta indica, Vernonia amygdalina and Gongronema latifolium for use as hop substitutes in the Nigerian beer industry has been studied. Examples of such studies abound. Okafor and Anichie in 1983 [32] showed that leaves of the vegetable, Gongronema latifolium (utazi) have great potential as substitute for hops in tropical beer brewing. It was found by the authors that this plant possessed some antiseptic properties against beer spoilage microorganisms. They also revealed that the chemical properties of beer brewed using this plant did not differ much from that brewed with hops though their organoleptic differences were pronounced. The authors
however did not characterize the vegetables as they only used it for brewing and sensory analysis.

In 1990, Adenuga et al. [33] brewed sorghum beer using extracts of *Gongronema latifolium*, *Vernonia amygdalina* and *Garcinia kola* to impart bitter taste and flavour as substitutes for hops. Sensory evaluation of the beers by trained panelists (connoisseurs) showed that the *Gongronema latifolium* flavoured beer was preferred to the other beers and compared favourably with hopped beer in terms of flavour and taste. In the same year, Anichie and Uwakwe [34] compared the chemical, brewing and anti-microbial properties of a tropical seed, *Garcinia kola* with traditional hops. The authors confirmed the presence of alpha acids in *Garcinia kola* though in lower concentration than hops but laboratory brewing trials gave beers with similar chemical properties. They also found that *Garcinia kola* beer was as acceptable to tasters as hopped beer except that it had an improved bitterness and the extracts (*Garcinia kola* and hop) exerted similar anti-microbial effects on two beer spoilage microorganisms (*Lactobacillus delbruckii* and *Candida vin*)

Ajebesone and Aina [35] in 2004 characterized four bitter plants used for beer in Africa: *Azadirachta indica*, *Garcinia kola*, *Gongronema latifolium* and *Vernonia amygdalina* to determine their potentiality as hop substitutes in beer production. They concluded that these Nigerian bitter vegetables can serve as substitutes for hops in tropical beer brewing based on their proximate analysis of the vegetables.

Our previous works between 2016 and 2017 [36-43] sought to find new sources of ingredients from some Nigerian bitter plants that can mimic hops and substitute them in beer brewing. We established that the vegetables may find application in the Nigerian beer industry as suitable hop substitutes but cannot categorically be concluded because we could not carry out shelf life, sensory and antimicrobial activities of our beer samples. For example, the results of the study on metabolites composition of *Garcinia kola* extract as potential substitute for isomerized hop extract in beer brewing [36] showed that *Garcinia kola* extract contains some metabolites comparable to those of isomerized hop extract, although some metabolites [dehydrcohumulonic 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 that of *Garcinia kola*. In comparative studies of the physicochemical properties of beers brewed with hop extracts and those from four selected tropical plants [38], the order of closeness of the extracts of the bitter plants investigated to that of isomerized hop was *G. Kola > G. latifolium > V. amygdalina > A. indica* while that to hop leaf extract was *G. Kola > G. latifolium > V. amygdalina > A. indica*. In the work, comparative evaluation of phytochemical constituents, bitterness characters and essential oil contents of extracts from four Nigerian plants as substitutes for hops [39], we concluded that the extracts from tested Nigerian plants could be used as suitable substitutes for hops in beer brewing based on the parameters investigated. Evaluation of antinutritional factors in hop extract and that of *A. indica* as potential hop substitute in the Nigerian beer industry [40] indicated that *A. indica* was closer to isomerized hop extract than to hop leaf extract in terms of antinutritional properties.

Okafor et al., 2020 [44] investigated the levels of polycyclic aromatic hydrocarbons (PAHs) in some beers brewed with hop, *Garcinia kola*, *Azadiricheta indica*, *Vernonia amygdalina* and *Gongronema latifolium*. They concluded that the extracts from the four Nigerian bitter vegetables could be used as potential substitutes for isomerized hop extract in the Nigerian brewing industry. The study also revealed the absence of the 16 priority EPA PAHs in all the beer samples except in the beer produced using extract from *Garcinia kola* where pyrene was detected which causes, on long term exposure cataracts, kidney and liver damages, jaundice, decreased immune function, breathing problems, asthma-like symptoms, lung function abnormalities, redness and skin inflammation, etc. The authors therefore recommended that the Nigerian brewery industry should not consider the substitution of isomerized hop extract with that from *Garcinia kola* in beer brewing since the consumption of beers brewed with this extract can undermine public health of the consumers. In a recent work by Okafor et al. [45] titled “gas chromatography-mass spectrometry profiling of hops and some Nigerian potential hop substitutes: comparative studies in beer brewing”, the authors found 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 as there were no alterations in the physicochemical properties of all their beer samples. Furthermore, it was concluded in our work [46] published in July, 2020 in America that the extracts from tested Nigerian plants could be used as suitable substitutes for hops in beer brewing with respect to their compared phytochemical constituents; extract of *G. latifolium* having the greatest potential as substitute for isomerized hop extract and that of *V. amygda* lina, the closest substitute for hop leaf extract.

*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 hepatotoxins [47]. *Garcinia kola* seeds are also used in the treatment of stomach ache, gastritis, diabetes, bronchitis and throat infections as well as treatment of liver disease and diarrhea [48-51]. *Azadirachta indica* is used in some parts of Nigeria for treatment of malaria. The plant is found to be antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative [52]. *Vernonia amygda* lina leaves are used as vegetable and to stimulate the digestive system, as well as reduce fever [53]. They are also used as local medium against leech which transmits bilharziasis [54,55]. *Gongronema latifolium* are widely consumed as vegetables. Different leaf extracts also showed moderate to promising antioxidant, anti-inflammatory, hepatoprotective, anti-plasmodial, anti-asthmatic, anti-sickling, anti-ulcer, analgesic, antipyretic, gastrointestinal relaxing, laxative and stomachic activities [56-59].

3.2 Chromatographic Techniques

In the furtherance of efforts for the substitution of imported hops with some Nigerian bitter plants, Okafor *et al.* conducted some research [44,45] using chromatographic techniques in the determination of active compounds in extracts from hops and Nigerian plants, and levels of polycyclic aromatic hydrocarbons (PAHs) contaminants in beers brewed with the extracts in which the authors adopted GC – MS and GC – FID techniques respectively.

![Chromatogram of isomerized hop extract](image)

**Fig. 4. Chromatogram of isomerized hop extract**

*(Okafor, 2016) [37]*
Fig. 5. Chromatogram of hop leaf extract
(Okafor, 2016) [37]

Fig. 6. Chromatogram of *G. kola* extract
(Okafor, 2016) [37]
Fig. 7. Chromatogram of *A. indica* extract
(Okafor, 2016) [37]

Fig. 8. Chromatogram of *V. amygdalina* extract
(Okafor, 2016) [37]
<table>
<thead>
<tr>
<th>S/N</th>
<th>Compound</th>
<th>Isomerized hop</th>
<th>Hop leaf</th>
<th>G. kola</th>
<th>A. indica</th>
<th>V. amygdalina</th>
<th>G. latifolium</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.</td>
<td>4,4-dimethyl-2-buten-4-olide</td>
<td>0.362</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
</tr>
<tr>
<td>5.</td>
<td>1,2-dimethyl-cyclopropane carboxylic acid</td>
<td>0.990</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
</tr>
<tr>
<td>6.</td>
<td>2,5-dimethyl-2-hexanol</td>
<td>0.268</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
</tr>
<tr>
<td>7.</td>
<td>Dehydro-cohumulonic acid</td>
<td>0.533</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
</tr>
<tr>
<td>8.</td>
<td>4,4,5,5-tetramethyl-bicyclo hexyl-6-ene-2,3-dione</td>
<td>0.925</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
</tr>
<tr>
<td>9.</td>
<td>1,2-benzenedicarboxylic, bis (-2-ethylhexyl) ester</td>
<td>0.114</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
</tr>
<tr>
<td>10.</td>
<td>Hexadecanoic acid</td>
<td>0.784</td>
<td>0.954</td>
<td>0.930</td>
<td>0.957</td>
<td>0.923</td>
<td>1.069</td>
</tr>
<tr>
<td>11.</td>
<td>Octadecenoic acid, methyl ester</td>
<td>0.369</td>
<td>0.314</td>
<td>0.284</td>
<td>0.434</td>
<td>0.512</td>
<td>0.077</td>
</tr>
<tr>
<td>12.</td>
<td>Octadecenoic acid, methyl ester</td>
<td>0.121</td>
<td>0.245</td>
<td>0.162</td>
<td>0.236</td>
<td>0.263</td>
<td>0.065</td>
</tr>
<tr>
<td>14.</td>
<td>Octadecanoic acid</td>
<td>1.792</td>
<td>2.556</td>
<td>2.331</td>
<td>2.458</td>
<td>2.406</td>
<td>Nf</td>
</tr>
<tr>
<td>15.</td>
<td>Hexadecanoic acid, 2-hydroxy -1,3-propanediyl ester</td>
<td>0.124</td>
<td>Nf</td>
<td>0.192</td>
<td>0.195</td>
<td>0.225</td>
<td>Nf</td>
</tr>
<tr>
<td>16.</td>
<td>9,12-octadecadienoic acid</td>
<td>0.465</td>
<td>0.126</td>
<td>0.104</td>
<td>0.715</td>
<td>Nf</td>
<td>0.795</td>
</tr>
<tr>
<td>17.</td>
<td>Octadecanoic acid, 2-hydroxyl -1,3-propanediyl ester</td>
<td>0.257</td>
<td>0.163</td>
<td>0.284</td>
<td>Nf</td>
<td>Nf</td>
<td>0.061</td>
</tr>
<tr>
<td>18.</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>Nf</td>
<td>0.113</td>
<td>0.069</td>
<td>0.141</td>
<td>0.146</td>
<td>Nf</td>
</tr>
<tr>
<td>19.</td>
<td>Lupulon (beta-lupulic acid)</td>
<td>Nf</td>
<td>0.202</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
</tr>
<tr>
<td>20.</td>
<td>Octadecanoic acid, oxiranyl</td>
<td>Nf</td>
<td>0.413</td>
<td>Nf</td>
<td>0.283</td>
<td>0.351</td>
<td>Nf</td>
</tr>
<tr>
<td>21.</td>
<td>methyl ester Hexadecanal</td>
<td>Nf</td>
<td>0.559</td>
<td>0.707</td>
<td>Nf</td>
<td>0.831</td>
<td>0.146</td>
</tr>
<tr>
<td>22.</td>
<td>2-methyl-3,13-octadecadienol</td>
<td>Nf</td>
<td>Nf</td>
<td>0.423</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
</tr>
<tr>
<td>23.</td>
<td>Hexadecanoic acid-2,3-dihydroxypropyl ester</td>
<td>Nf</td>
<td>Nf</td>
<td>0.104</td>
<td>0.085</td>
<td>Nf</td>
<td>Nf</td>
</tr>
<tr>
<td>24.</td>
<td>Benzoic acid, 2-(aminocarbonyl)</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>0.298</td>
</tr>
<tr>
<td>25.</td>
<td>Octadecanoic acid, 2-(2-hydroxyethoxy) ethylester</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>2.546</td>
</tr>
<tr>
<td>26.</td>
<td>9,12-octadecadien-1-ol</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>Nf</td>
<td>0.491</td>
</tr>
</tbody>
</table>

*Nf= Not found; (Okafor, 2016) [37]
3.2.1 Profiling of compounds of the extracts

GC-TOFMS is a benchmark approach for metabolomics data acquisition from chromatographic peaks [60]. 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 [61]. 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 chemical compound library of simulated mass spectral information [62]. Chromatograms (Figs. 4-9) for imported hop extracts and those from four Nigerian bitter plants had been obtained and spectrum matching achieved by programming the software to compare the chromatogram of the mass spectra to simulated library peaks [63].

3.2.2 Deconvolution and elucidation of compounds of the extracts

Table 3 shows clearly the compounds and their abundance in the extracts as acquired from Figs. 4-9. Figs. 10-15 (compound, formula and structure) show that a total of twenty-three (23) organic compounds were identified in all the extracts. 4,4-dimethyl-2-buten-4-olide (4); 1,2-dimethyl cyclopropane carboxylic acid (5); 2,5-dimethyl-2-hexanol (6); dehydro-columulinc acid (7); 4,4,5,5-tetramethyl-bicyclo-hexyl-6-ene-2,3-dione (8) and 1,2-benzendicarboxylic acid, bis (2-ethylhexyl) ester (9) were found in isomerized hop extract. Lupulone (19), a beta- acid was present in only hop leaf extract. These compounds found in hops were absent in the Nigerian bitter vegetables. The presence of the same chemical moieties in different extracts is an indication that the extracts can mimic one another. In this case, extracts from Nigerian bitter plants show divergent chemical moieties from imported hops. This is an indication that none of the Nigerian bitter plants could be used perfectly as a suitable substitute for hops in beer brewing. However, hexadecanoic acid (10), octadecenoic acid methyl ester (11), octadecanoic acid methyl ester (12) and octadeconoic acid (13) were found in all the extracts. Compound (14) was found in the extracts of hop leaf, isomerized hop, G. kola, A. indica and V. amygdalina. 9,12-Octadecadienoic acid (16) was found in the extracts of isomerized hop, hop leaf, G. kola, A. indica and G. latifolium. Octadecanoic acid, 2-hydroxy-1, 3-propandiyi ester (17) was found in the extracts of isomerized hop, hop leaf, G. kola, and G. latifolium. Another significant observation...
is that each extract of hop leaf, *G. kola*, *V. amygda**lina* and *G. latifolium* contained 9-hexadecenal (21) which was absent in extracts of isomerized hop and *A. indica*. Also, each extract of hop leaf, *G. kola*, *A. indica* and *V. amyd**galina* contained hexadecanoic, methyl ester (18) that was not present in both the extracts of isomerized hop and *G. latifolium*. Again, there are compounds which were present in the local substitutes that were conspicuously absent in imported hops even though the Nigerian plants contained these constituents differently, e.g. while *G. kola* alone contained 2-methyl-3,13-octadecadiene-1-ol (22), *G. latifolium* alone contained compounds Octadecanoic acid 2-(2-hydroxy-ethoxy) ethyl ester (25) and 9,12-Octadecadien-1-ol (26). All these compounds were completely absent in imported hops. These differences and similarities in the constitution of chemical compounds in the local plants and those of imported hops may explain the reason why the organoleptic character of beers brewed with imported hops and those brewed with *G. latifolium* by Okafor and Anichie [32] in 1983 were more pronounced while their chemical properties did not differ much. On the basis of these observations, it can be said that chemical compounds present in imported hops and those in the Nigerian plants differed significantly and hence, these local plant extracts could not be used as suitable substitutes for hops in beer brewing.
Octadecanoic acid, 2-hydroxy-1, 3-propandiy1 ester

(17) $C_{39}H_{78}O_5$

Fig. 10. Compounds of isomerized hop extract

Hexadecanoic, methyl ester

(18) $C_{17}H_{34}O_2$

Hexadecanoic acid

(10) $C_{18}H_{32}O_2$

Octadecenoic acid, methyl ester

(11) $C_{19}H_{38}O_2$

Octadecanoic acid, methyl ester

(12) $C_{19}H_{38}O_2$

Octadecanoic acid

(13) $C_{18}H_{34}O_2$

Octadecanoic acid

(14) $C_{18}H_{36}O_2$

9, 12-Octadecadienoic acid

Octadecanoic acid, 2-hydroxy-1, 3-propandiy1 ester

Lupulone (beta-lupulic acid)

(16) $C_{19}H_{32}O_2$

(17) $C_{39}H_{78}O_5$

(19) $C_{26}H_{38}$

Octadecanoic acid, oxiranyl methyl ester

(20) $C_{21}H_{40}O_3$

9-Hexadecenal

(21) $C_{16}H_{32}O$

Fig. 11. Compounds of hop leaf extract

Hexadecanoic, methyl ester

(18) $C_{19}H_{32}O_2$

Hexadecanoic acid

(10) $C_{19}H_{38}O_2$

Octadecenoic acid, methyl ester

(11) $C_{17}H_{34}O_2$
Fig. 12. Compounds of G. kola extract

Octadecanoic acid, methyl ester
(12) C_{19}H_{38}O_2

Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester
(15) C_{35}H_{68}O_5

Octadecanoic acid
(14) C_{18}H_{36}O_2

Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester
(15) C_{35}H_{68}O_5

9,12-Octadecadienoic acid
(16) C_{18}H_{32}O_2

Hexadecanoic acid
(18) C_{17}H_{34}O_2

Octadecenoic acid, methyl ester
(10) C_{18}H_{32}O_2

Hexadecanoic acid
(17) C_{39}H_{76}O_5

Hexadecenoic acid
(11) C_{19}H_{38}O_2

Octadecanoic acid, methyl ester
(12) C_{19}H_{38}O_2

Octadecenoic acid
(13) C_{18}H_{34}O_2

Octadecanoic acid
(17) C_{39}H_{76}O_5

Hexadecanoic acid, 2,3-dihydroxypropyl ester
(23) C_{19}H_{38}O_4

2-Methyl-3,13-octadecadiene-1-ol
(22) C_{19}H_{36}O

Hexadecenoic acid
(19) C_{18}H_{36}O_4

Hexadecanoic, methyl ester
(18) C_{17}H_{34}O_2

Hexadecanoic acid
(10) C_{18}H_{32}O_2

Octadecenoic acid, methyl ester
(12) C_{19}H_{38}O_2

Octadecanoic acid
(14) C_{18}H_{36}O_2

Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester
(15) C_{35}H_{68}O_5

9,12-Octadecadienoic acid
(16) C_{18}H_{32}O_2
Octadecanoic acid, oxiranylmethyl ester

(20) C_{21}H_{40}O_{3}

Hexadecanoic acid, 2, 3-dihydroxypropyl ester

(23) C_{19}H_{38}O_{4}

Fig. 13. Compounds of *A. indica* extract

Hexadecanoic acid, methyl ester

(18) C_{17}H_{34}O_{2}

Hexadecanoic acid

(10) C_{16}H_{32}O_{2}

Octadecenoic acid, methyl ester

(11) C_{19}H_{36}O_{2}

Octadecanoic acid, methyl ester

(12) C_{19}H_{38}O_{2}

Octadecenoic acid

(13) C_{18}H_{34}O_{2}

Octadecanoic acid

(14) C_{18}H_{38}O_{2}

Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester

(15) C_{35}H_{68}O_{5}

Octadecanoic acid, oxiranyl methyl ester

(20) C_{21}H_{40}O_{3}

Hexadecenal

(21) C_{16}H_{30}O

Fig 14. Compounds of *V. amygdalina* extract

Benzoic acid, 2-(aminocarbonyl)

(24) C_{8}H_{7}NO_{3}

Hexadecanoic acid

(10) C_{16}H_{32}O_{2}

Octadecenoic acid, methyl ester

(11) C_{19}H_{38}O_{2}

Octadecanoic acid, methyl ester

(12) C_{19}H_{38}O_{2}

Octadecanoic acid

(13) C_{18}H_{34}O_{2}

Octadecanoic acid 2-(2-hydroxyl-ethoxy) ethyl ester

(25) C_{22}H_{42}O_{2}
3.2.3 Identification and quantification of polycyclic aromatic hydrocarbons

Beer samples brewed with extracts from Nigerian bitter plants and in comparison with a control have been analysed. Methodology for such analysis had been published [43, 64-67].

Fig. 15. Compounds of G. latifolium extract

Beer samples brewed with extracts from Nigerian bitter plants and in comparison with a control have been analysed. Methodology for such analysis had been published [43, 64-67].

Fig. 16 shows 16 United States of American Environmental Protection Agency (USEPA) priority PAHs (compounds 27-42) with their chemical structures and formulas while Figs. 17-21 show the GC-FID Chromatograms (fingerprints) of extracts from the plant species.

Identification and quantification of individual PAHs in the beer samples using the chromatograms show that pyrene was detected in beer brewed with Garcinia kola extract and no other PAHs was detected in all the beer samples [44].

Fig. 16. Chemical structures and formulas of the 16 priority USEPA PAHs
Fig. 17. Chromatogram of Star lager beer (Okafor et al. [44])

Fig. 18. Chromatogram of beer produced with *G. kola* extract (Okafor et al. [44])

Fig. 19. Chromatogram of beer produced with *A. indica* extract (Okafor et al. [44])
Fig. 20. Chromatogram of beer produced with *V. amygdalina* extract (Okafor et al. [44])

Fig. 21. Chromatogram of beer produced with *G. latifolium* extract (Okafor et al. [44])

Table 4. Retention time of the beers

<table>
<thead>
<tr>
<th>PAH</th>
<th>Isomerized hop</th>
<th>G. <em>kola</em></th>
<th>A. <em>indica</em></th>
<th>V. <em>amygdalina</em></th>
<th>G. <em>latifolium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>10.486</td>
<td>10.486</td>
<td>10.486</td>
<td>10.486</td>
<td>10.486</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>12.228</td>
<td>12.228</td>
<td>12.228</td>
<td>12.228</td>
<td>12.228</td>
</tr>
<tr>
<td>Anthracene</td>
<td>12.305</td>
<td>12.305</td>
<td>12.305</td>
<td>12.305</td>
<td>12.305</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>17.539</td>
<td>17.539</td>
<td>17.539</td>
<td>17.539</td>
<td>17.539</td>
</tr>
<tr>
<td>Chrysene</td>
<td>17.638</td>
<td>17.638</td>
<td>17.638</td>
<td>17.638</td>
<td>17.638</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>20.057</td>
<td>20.057</td>
<td>20.057</td>
<td>20.057</td>
<td>20.057</td>
</tr>
<tr>
<td>Dibenzo[a, h]anthracene</td>
<td>23.183</td>
<td>23.183</td>
<td>23.183</td>
<td>23.183</td>
<td>23.183</td>
</tr>
<tr>
<td>Indeno[1, 2, 3-c, d]pyrene</td>
<td>23.247</td>
<td>23.247</td>
<td>23.247</td>
<td>23.247</td>
<td>23.247</td>
</tr>
<tr>
<td>Benzo[g, h, ij]perylene</td>
<td>23.673</td>
<td>23.673</td>
<td>23.673</td>
<td>23.673</td>
<td>23.673</td>
</tr>
</tbody>
</table>

Source: Okafor et al. [44]
Table 5. Concentration of the 16 priority EPA PAHs in the samples

<table>
<thead>
<tr>
<th>PAH</th>
<th>Concentration (mg/kg)</th>
<th>Isomerized hop</th>
<th>G. kola</th>
<th>A. indica</th>
<th>V. amygdalina</th>
<th>G. latifolium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Fluorene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Anthracene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Fluoranthrene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Pyrene</td>
<td>Nd</td>
<td>0.00402</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Chrysene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Benzo[b]fluoranthrene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Benzo[k]fluoranthrene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Dibenzo[a, h]anthracene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Indeno[1, 2, 3-c, d]pyrene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Benzo[g, h, i]perylene</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
</tbody>
</table>

Nd = Not detected; Source: Okafor et al. [44]

4. OVERVIEW

It has been established that the Nigerian bitter plants could be used as suitable substitutes for hops in the Nigerian beer industry with respect to physicochemical properties, proximate composition, phytochemical constitution, antimicrobial activities etc. but in terms of composition of chemical moieties and organoleptic characters or sensory properties, the Nigerian bitter plants were found to be unsuitable for use as potential substitutes in the beer industry. Moreover, in the determination of levels of PAHs in beers brewed with hop and the bitter plants, substitution with *Garcinia kola* was faulted because pyrene was detected in the beer produced with *Garcinia kola* extract. The significance of the determination of PAHs is reflected by the special attention the European Union is paying to regulating their maximum allowed levels in various types of foodstuffs and beverages. Like tobacco and smoked meats, alcoholic drinks can also contain these carcinogenic chemicals, as the latter have been detected in the charred insides of barrels, some ingredients such as caramel or the smoke released during the drying of germinated barley in beer or whisky [68]. However, the authors in the work, levels of PAHs in beers brewed with hop and the Nigerian bitter plants [44] explained the presence of pyrene in the beer sample brewed with extract of *G. kola* from plantation point of view asserting that the *G. kola* used in their work may have been harvested from a refuse dumpsite since refuse dumpsites had been reported as a candidate source of PAHs [69].

5. CONCLUSIONS AND RECOMMENDATIONS

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. Chemical compounds present in imported hops and the Nigerian plants differed significantly. Imported hops contained seven compounds that were not found in the Nigerian plants. Four compounds were found to be present in both hop leaves/processed female hop inflorescence and the Nigerian plants. There were also some compounds which were present in the Nigerian plants but absent in imported hops. The study also revealed the absence of the 16 priority USEPA PAHs in all the beer samples except in the beer produced using extract from *G. kola* where pyrene was detected which causes, on long term exposure cataracts, kidney and liver damages, jaundice, decreased immune function, breathing problems, asthma-like symptoms, lung function abnormalities, redness and skin inflammation, etc. and therefore academic activity in the area of mixtures/blends of extract of plant species which mimic hop taste is strongly recommended.
DISCLAIMER

The products named and used for this review are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the review was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT

The authors acknowledge Professor V. I.E. Ajiwe of the Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria for proofreading the entire manuscript and making some useful suggestions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

45. Okafor VN, Okafor UW, Anyalebechi IR. Gas chromatography-mass spectrometry (GC-MS) profiling of hops and some


65. Fayemi AA. The processing and preservation of bitter leaf (Vernonia amygdalina). M.Sc. thesis in the Department of Food Technology University of Ibadan, Nigeria; 1982.


© 2020 Okafor et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/60661