AJMHR
Asian Journal of Medical and Health Research
Journal home page: www.ajmhr.com

Comparative Studies of Bitterness, Phytochemical and Mineral Contents of Hop Extracts and Extracts from Four Selected Tropical Plants

Vincent Nwalieji Okafor[1]*, Augustine Nnalue Eboatu[1], Patrick Enuneku Omuku[1]
1.Department of Pure & Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria

ABSTRACT

The potential of four selected Nigerian plants namely Azadirachta indica (neem), Garcinia kola (bitter cola), Gongronema latifolium (heckel) and Vernonia amygdalina (bitter leaf) as substitutes for hops in beer brewing were evaluated in terms of methanolic extracts. Bitterness character of the extracts was investigated using UV-visible spectrophotometer. Phytochemical assays of the extracts were carried out using standard methods. Mineral contents of all the extracts were carried out using Atomic Absorption Spectrophotometer (AAS) after digestion with perchloric acid and concentrated nitric acid. These tropical plants were statistically ranked by the application of Analysis of Variance (ANOVA). The concentration of iso-alpha acid ranged from 7.95-12.53ppm. Phytochemical assay revealed that alkaloid content in all the extracts ranged between 3.2-4.8%; tannin ranged from 2.0-4.8% and saponin ranged from 0.80-5.20%. The AAS results showed that the concentration of metals investigated in all the samples were calcium (16.300–33.145ppm), sodium (92.019–101ppm), potassium (8.297–206.838ppm), magnesium (19.331-22.188ppm), lead (Not detected), manganese (0.426-38.628ppm), cobalt (0.00–0.002ppm), zinc (0.963-17.944ppm), mercury (0.00–1.127ppm) and iron (0.159-8.614ppm). It was established from ranking that the order of closeness of the vegetables investigated to isomerized hop extract was G. latifolium (0.919) > G. kola (0.819) > A. indica (0.712) > V. amygdalina (0.517) while that to hop leaf extract was V. amygdalina (0.964) > G. kola (0.679) > G. latifolium (0.433) > A. indica (0.288). Hence, the extracts from tested Nigerian plants could be used as suitable substitutes for hops in beer brewing. Extract of G. latifolium had the greatest potential as substitute for isomerized hop extract and that of V. amygdalina was the closest substitute for hop leaf extract.

Keywords: hops, extract, mineral, phytochemicals, iso-alpha acid, Nigerian plants, substitutes, bitterness.

*Corresponding Author Email: vinokafor@yahoo.com
Received 20 August 2016, Accepted 21 September 2016

Please cite this article as: Okafor .VN, Comparative Studies of Bitterness, Phytochemical and Mineral Contents of Hop Extracts and Extracts from Four Selected Tropical Plants. Asian Journal of Medical and Health Research 2016.

INTRODUCTION

The bitterness in beer is caused mainly by iso-alpha acids of hops and marginally by phytochemicals such as tannins and alkaloids. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are natural bioactive compounds found in plant food, leaves, seeds, roots and stems. Most phytochemicals are known to possess many properties which make them vital to both plants and animals. Some of these properties are antioxidant, anti-microbial and physiological activities. Phytochemicals are present in hops.

Hops, an essential 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 (Ashurst, 1971)[1].

The hop (*Humulus lupulus L)* is a perennial dioecious climbing plant of hemp (*cannabis)* family and belonging to the order (*urticales*) which also includes the nettle family (Kunze, 1999)[3]. Hop plants are vital to the brewing industry and some of their unique chemicals have the potential to be used in the nutraceutical industry (Shellie *et al*., 2009)[2]. Only two species of *Humulus* are recognized: *Humulus lupulus L.* (*H. americanus, H. neomexicanius* and *H. cordifolius)* and *H. japonicas* sieb. The latter is an annual ornamental climbing plant from Japan devoid of resin and therefore of no brewing value. The genus *Humulus* is included in the natural family *Cannabinaceae* together with *cannabis*, which is only represented by *C. sativa* (Indian hemp, marihuana or hashish). Chemical similarities are seen between *H. lupulus* and *C. sativa* but the resins of the two species are completely distinct. Those of the hop provide the bitter principles of beer whilst those of the *cannabis* include the psychotomimetic principles of drug (Crombie and Crombie, 1975)[4].

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 (Burgess, 1964)[5].

The brewing value of the hop is found in its resins and essential oils. Peacock (2009)[6] put it that the brewing value of the hop is found in hop resins and essential oils that are contained in the lupulun 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 (Hudson, 1970)[7].

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.

Hop resins are sub-divided into hard and soft based on their solubility. 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 (Westwood, 1994)[8].

The three major components of alpha acids are humulone, cohumuloe and adhumulone (Hough *et al*., 1982)[9].

Alpha acids are also 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 (Hough *et al*., 1982; Koller, 1969)[9,10]. In this conversion, humulone is converted to iso-humulone, cohumulone to iso-cohumulone and adhumulone to iso-adhumulonme.

Hops are grown throughout the temperate regions of the world to meet the demands of the brewing industries (Hough *et al*., 1982)[9]. Nigeria is in tropical region but beer production in Nigeria has increased recently due to ready markets and the importation of hops to meet the demand of the brewing industries becomes inevitable and continues to constitute a significant proportion of the Nigerian economy. Consequently, huge amounts of foreign exchange are being spent by this sector on importation of hops.

A lot of efforts have been made in the brewing industry for the substitution of barely with some local cereals. The substitution of hops with local raw materials has not received commensurate attention.

This piece of work was designed to identify the precursors of bitterness of hops and investigate quantitatively some phytochemical and mineral (metal) contents of hops as well as contrast to the four selected Nigerian plants. It was also designed to find the possibility of these plants serving as substitutes for hops in beer production. This level of raw material freedom confers definite economic advantages to the Nigerian brewing industry.

Vernonia amygdalina and Gongronema latifolium are widely consumed as vegetable in Nigeria. *Azadirachta indica* is used in some parts of Nigeria for treatment of malaria while *Garcia kola* is used in some areas for the treatment of stomach ache and gastritis (Iwu, 1990; Okpoko, 2010; Akuodor, 2010; Joshi, 2011)[11-14]. One thing they have in common with hop is that they are bitter like hop but thrive in tropical regions, unlike hop (Ajebesone and Aina, 2004)[15].

MATERIALS AND METHOD

Procurement of Materials

Hop leaf and isomerised hop extract were respectively purchased from Youngs Ubrew Goldings Hops and Ritchies both in the United Kingdom. The leaves of *A. indica*, *G. latifolium*, *V. amygdalina* and the seeds of *G. kola* were obtained from the herbarium of Nnamdi Azikiwe University, Awka. Chemicals used were as detailed by AOAC, ASBC, and IOB.

Methods

Sample Preparation

Except for the isomerized hop extract prepared by Ritchies, each plant sample was milled and vacuum dried at 50^{o}C. Two kilograms (2kg) of each plant material thus prepared was stored in a dessicator for the rest of the experiment. Three hundred grams (300g) each of the resulting powders were then used to obtain the extracts by steeping procedure.

Methanol Extraction

The methanol extract was prepared by steeping 300g of the dry powdered plant material in 1.5 litres of methanol at room temperature in a tight fitting round bottom flask for forty eight hours. The mixture was filtered first through a Whatman filter paper (No. 42) and then through a sintered glass funnel. The filtrate was concentrated using a rotary evaporator with water bath set at 40^{o}C for 2 hours to obtain each extract. The extract was stored in amber coloured reagent polypropylene bottle in a deep freezer (Thermofrost, Mod.TR150S) at -5^{o}C for subsequent analysis.

Alpha Acid

Two (2) grams of the extract was acidified with 10 ml of 0.002M HCl and its absorbance read at 355nm, 325nm and 275nm respectively using a spectrophotometer. The spectrophotometer was switched on and calibrated using a blank (pure methanol). The sample was then inserted into the curette and the absorbance read at 275nm and recorded. The curette was then rinsed. The procedure was repeated and the absorbance read at 325nm and 355nm respectively. The alpha acid was computed as follows:

Concentration of alpha acid = $73.79A_{325}$- $51.56A_{355}$ – $19.07A_{275}$ mg/l where A = absorbance at the specified wavelength (Hough *et al*., 1982)[9].

Tannins

To determine tannin, the Butler (1989)[16] titration method as described by Viji and Parvatham (2011)[17] was adopted. To 2.5g of the extract in a conical flask was added 100cm^{3} of petroleum ether and stoppered for 24 hours. The solution was thereafter filtered and allowed to stand for further 15 minutes in the open for the ether to evaporate. It was thereafter re-

extracted with 100cm^3 of 10% acetic acid in ethanol for 4 hours. The solution was then filtered and the filtrate collected. Twenty five ml of concentrated NH_4OH was added to the filtrate to expel any alkaloid that may interfere by precipitation. The solution was filtered again and the filtrate was heated with electric hot plate to remove some of the NH_4OH still in the solution. The heating continued until the volume was 33cm^3. To 5cm^3 of this solution was added 20cm^3 of ethanol. The mixture was titrated with 0.1M NaOH using phenolphthalein as indicator until pink end point was reached. The tannin content was then calculated in percentage as follows:

$C_1V_1 = C_2V_2$ where

C_1 = Conc. of tannic acid (sample concentration)

C_2 = Conc. of base = 0.1M NaOH

V_1 = Volume of tannic acid = 5cm^3 (sample volume)

V_2 = Volume of base = Average titre value

Therefore,

$$\% \text{ tannic acid content} = \frac{C_1 \times 100}{\text{Weight of sample analyzed}}$$

Alkaloids

2.5g of the methanolic extract was weighed into a 250cm^3 beaker and 200cm^3 of 20% acetic acid in ethanol was added and covered, and allowed to stand for four hours at room temperature. This was filtered with Whatman No. 42 filter paper. The filtrate was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until precipitation occurred, followed by filtration through a pre-weighed sintered glass funnel and subsequently washed with 0.2M ammonia solution. The residue on the sintered glass is the alkaloid which is dried in the oven at 80^{o}C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed (Harbone, 1995)[18].

$$\% \, Alkaloid = \frac{Weight\ of\ sintered\ glass\ funnel\ with\ residue - Weight\ of\ sintered\ glass}{Weight\ of\ sample\ analyzed} \times 100$$

Saponins

2.5g of the extract was dissolved in 250cm^3 20% acetic acid in ethanol in a 500cm^3 beaker and allowed to stand in a water bath at 50^{o}C for 24 hours. This was filtered and the filtrate was concentrated using a water bath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the filtrate until precipitation was complete. The solution

was filtered with a pre-weighed sintered glass funnel and dried in an oven at 105^{o}C for 30 minutes. The Saponin content was calculated in percentage (Harbone, 1995)[18].

$$\%\ Saponin\ Content = \frac{weight\ of\ sintered\ glass\ funnel\ with\ precipitate - weight\ of\ sintered\ glass\ funnel}{Weight\ of\ sample\ analyzed} \times 100$$

Mineral Analysis

2g of the extract contained in a 250cm^3 beaker was added 10cm^3 of perchloric acid and 10cm^3 of concentrated HNO_3. This was boiled on a hot plate in a fume cupboard till white fumes started evolving. The digesate was further recharged by the digesolve and heated till white fumes were given off. This was followed by addition of 20cm^3 of deionized water. Boiling was continued for a further 20 minutes till the mixture became particle less. The digested sample was brought down and cooled under hood, to room temperature. It was subsequently filtered through a No. 11 Whatman filter paper and the filtrate collected in a 50cm^3 volumetric flask. 20cm^3 of deionized water was used to rinse the filter paper before the combined filtrate was made up to mark, and poured into a sample container, labeled 'ready for AAS analysis'.

Standards were prepared from the salts of the metals to be analyzed and relevant lamps were fixed for the analysis. This was done for calcium, sodium, potassium, magnesium, cobalt, mercury, lead, iron, zinc and manganese. The diluents of sample were aspirated into the Atomic Absorption Spectrophotometer using the filter corresponding to each mineral element.

Statistical Analysis

In the test of significant difference, One Way Analysis of Variance (ANOVA) is the most suitable tool as it has the capacity to show the existence of difference at 5% level of significance (Gupta, 2011)[36]. In ANOVA, two hypotheses, H_0 and H_1 are stated and tested for:

H_0; there is no significant difference among samples of interest.

H_1; 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

Iso- alpha acid

Iso- alpha acid content in all the samples ranged between 7.95 and 12.53ppm with isomerized hop extract having the highest iso- alpha acid of 12.53ppm and hop leaf extract, the lowest

iso- alpha acid of 7.75ppm. Table1 shows that the concentration of iso-alpha acid in all extracts were comparable. The result of iso-alpha acid in *V. amygdalina* is in agreement with that obtained by Adama *et al*. (2011)[19] in their investigation of bitter leaf as local substitute for hops in the Nigerian brewing industry.

Table 1: Iso-alpha Acid of the Extracts

Extract	Iso-alpha acid (ppm)
Isomerized hop	12. 53
Hop leaf	9.75
G. kola	8.78
A. indica	10.12
V. amygdalina	9.44
G. latifolium	9.67

These results are consistent with the report of Ashurst (1971)[1] that non-polar fat solvents are suitable for the bittering constituents in hops and that bitterness level in beers depends on the age and method of storage of hops used in brewing.

Tannins

Tannin was present in all the samples but highest in *V. amygdalina* with 4.8% and lowest in Hop leaf with 2.0%. Table 2 shows the tannin content of the various samples. It can be seen that *V. amygalina* and *G. latifolium* contained 4.8% and 4.4% tannin respectively. *A. indica* contained 4.0% while *G. kola* and isomerized hop contained 2.8% and 3.6% respectively. Hence, tannin content was somewhat comparatively uniform in all the samples except in Hop leaf and *G. kola* and thus all the local vegetables except *G. kola* could substitute hops, if the volumes of their extracts are somehow reduced during hopping.

Table 2: Tannin content in the samples

Sample	Tannin (%)		
	Mean	St Dev	Range
Isomerized hop	3.6	0.300	0.6
Hop leaf	2.0	0.173	0.3
G.kola	2.8	0.265	0.5
A. indica	4.0	0.200	0.4
V. amygdalina	4.8	0.458	0.9
G. latifolium	4.4	0.361	0.7

Tannins (commonly referred to as tannic acids) are polyphenols present in many plant foods that form colloidal solution in water (Buttler and Bailey, 1973)[20]. These solutions have astringent (mouth puckering) taste. Tannins are involved in the formation of haze in beer and also contribute to its taste and colour.

Tannins have been reported (Siddiqui and Ali, 1997)[21] to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional

value. However, the anticarcinogenic and antimutagenic potentials of tannins have been reported to be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation (Singh and Sastri, 1981)[22].

Alkaloids

The alkaloid contents of the samples studied are shown in Table 3. All the samples contained alkaloids. Alkaloid content was highest in *V. amygdalina* with 4.8% and lowest in isomerized hop extract with 3.2%. All the other samples contained equal percentages of alkaloids. On the basis of this alone, any of the local raw materials could be a suitable substitute for hops.

Table 3: Alkaloid content in the samples

Sample	Alkaloid (%)		
	Mean	St Dev	Range
Isomerized hop	3.2	0.265	0.5
Hop leaf	4.0	0.300	0.6
G.kola	4.0	0.436	0.8
A. indica	4.0	0.173	0.3
V. amygdalina	4.8	0.346	0.6
G. latifolium	4.0	0.173	0.3

Alkaloids are heterogeneous group of naturally occurring compounds found in plants. Some stimulate the nervous system; others can cause paralysis, elevate blood pressure or lower it and certain alkaloids act as pain relievers and as tranquilizers while others have been noted to contain antimicrobial properties (Hammer *et al*., 1999; Bandyophadhay *et al*., 2002; Parek *et al*., 2005)[23-25].

Saponins

Except *A. indica* that contained the highest saponin content of 5.2%, isomerized hop, hop leaf and *G. latifolium* were comparable in saponin contents. *V. amygdalina* had the lowest, followed by *G. kola* (Table 4).

Table 4: Saponin content in the samples

Sample	Saponin (%)		
	Mean	St Dev	Range
Isomerized hop	2.8	0.458	0.9
Hop leaf	3.2	0.346	0.6
G.kola	1.2	0.265	0.5
A. indica	5.2	0.436	0.8
V. amygdalina	0.8	0.173	0.3
G. latifolium	2.4	0.500	1.0

These factors showed that *G. latifolium* could substitute imported hops. If the volume of *G. kola* is doubled, that of *A. indica* halved, and *V. amygdalina* increased thrice, then, they could substitute imported hops as far as Saponin content is concerned

Saponins are steroidal glycosides that foam in water. They contribute to foam formation in beer and therefore have been reported to be helpful in reducing cholesterol during treatment of heart problems, and in building body structure (Akerele, 1993)[26].

MINERAL CONTENT

It is evident from Table 5 that this metal is available in all the samples. It shows that *G. latifolium* could substitute imported hops in beer brewing since their concentrations did not differ much. In a like manner, *G. kola*, *A. indica* and *V. amygdalina* can substitute one another. However, if the concentrations of *G. kola*, *A. indica* and *V. amygdalina* are halved during hopping, then, they could substitute imported hops. Calcium ion is by far the most influential mineral in the brewing process. Calcium reacts with phosphates forming precipitates leading to the release of hydrogen ions and in turn lowering of the pH of the mash. This lowering of the pH is critical because it provides an environment for alpha-amylase, beta amylase and proteolytic enzymes (Bamforth, 2006)[27]. Calcium is required by humans to perform some of the metabolic functions like nerve transmission, intracellular signaling and hormonal secretion, and providing structure and strength to bones and teeth.

The concentration of sodium was virtually the same range for all the samples (Table 5) especially isomerized hop, *G. latifolium*, *A. indica*, *V. amygdalina* and *G. kola*. Sodium plays a major role in controlling blood pressure and blood volume, for proper functioning of muscles and nerves. However, sodium has no chemical effect in beer but it contributes to the perceived flavour of beer by enhancing its sweetness levels from 75ppm to 150ppm, gives round smoothness and accentuates sweetness, which is most important when paired with chloride than when associated with sulphate ions (Goldamer, 2008)[28]. In the presence of sulphate, sodium creates an unpleasant harshness.

The concentration of potassium in isomerized hop, *A. indica* and *G. latifolium* were comparatively close. The concentrations of potassium in *G. kola* and *V. amygdalina* did not differ much while Hop leaf had the least concentration of potassium as shown in Table 5. Potassium is one of the important minerals the body needs to form proteins and muscles, maintain normal growth of the body, control electrical activity of the heart and help in various metabolic processes (Drake, 2010)[29].

Like sodium, potassium can create a 'Salty' flavour effect in beer. It is required for yeast growth and inhibits certain mash enzymes at concentrations above 10mg/L (Sanchez, 1999)[30]. Hence, *G. latifolium* and *A. indica* could substitute isomerized hop.

Like the case of calcium, magnesium is a very useful metal and an essential mineral to the body that helps to form proteins, produce and transport energy, maintain proper functioning of certain enzymes, and contract and relax muscles. Magnesium ions react similarly to

calcium ions, but since magnesium salts are much more soluble, the effect on wort pH is of little consequence. Magnesium carbonate reportedly gives more astringent bitterness than calcium carbonate (Stewart and Russel, 1985)[31]. Calcium and magnesium chlorides give body, palate fullness, and soft sweet flavour to beer. From Table 5, magnesium occurred comparably in all the samples. Thus, each can substitute the other in beer production.

Lead is a highly toxic metal. It can injure the kidney and cause symptoms of chronic toxicity, including impaired kidney function, hepatic dysfunction and poor reproductives. Moreover, lead can cause reduced intelligence quotient, learning difficulties, slow growth, behavioral abnormalities, hearing difficulties and cognitive functions in humans (Donaldin *et al*, 2008)[32]. From this work, lead is absent in all the samples as expected. Thus, each can substitute the other

Table 5: Mineral Content of the Extracts

Samples	Metal Concentration (ppm)									
	Ca	Na	K	Mg	Pb	Mn	Co	Zn	Hg	Fe
Isomerized hop	16.3	98.245	206.838	19.331	0	0.426	0.012	0.963	0	0.547
Hop leaf	17.8	92.019	8.297	21.113	0	0.85	0.008	1.985	0	0.815
G. kola	33.145	100.151	82.737	21.586	0	1.038	0.002	6.072	0	1.62
A. indica	33.145	95.122	206.838	20.971	0	0.667	0.019	1.611	1.127	2.526
V. amygdalina	33.717	101.263	60.24	20.24	0	0.782	0	1.09	0.39	0.159
G. latifolium	18.4	95.882	206.838	22.188	0	38.628	0.004	17.944	0	8.614

Manganese was virtually absent in all the samples except in *G. latifolium* where it occurred with some prominence. Thus, the other samples could substitute one another. Manganese is a mineral element that is both nutritionally essential and potentially toxic. Manganese plays an important role in a number of physiological processes as a constituent of multiple enzymes and as an activator of other enzymes; for example, wound healing is a complex process that requires increased production of collagen. Manganese is required for the activation of prolidase, an enzyme that functions to provide the amino acid, proline, for collagen formation in human skin cells (Higdon, 2001).[33]

Cobalt was somewhat high in *A. indica* but comparatively close in concentrations in isomerized hop and hop leaf. *G. latifolium* and *G. kola* respectively contained 0.004ppm and 0.002ppm while this metal was absent in *V. amygdalina*. On the basis of these observations, none of the Nigerian bitter vegetables can substitute imported hops but when the quantity of *A. indica* is reduced by half it can then substitute imported hops. Cobalt as a metal is known to be beneficial to mammals at low concentrations and toxic at elevated concentrations. Cobalt is part of the vitamin B_{12} molecule as cobalmin. The functions and activity of cobalt are essentially the same as vitamin B_{12}. Therefore, cobalt plays a role in erythropoiesis. However, industrial exposure to high amounts of cobalt and consumption of beer

contaminated with excessive amounts of cobalt produce cardio – myopathy with high mortality risks (http//www.vitamineherbuniversity.com/ topic.asp? categoryid=2&topics).

The concentrations of zinc in hop leaf and *A. indica* were comparatively uniform. It was especially high in *G. latifolium* and least in Isomerized hop. *G. kola* and *V. amygdalina* contained 6.072ppm and 1.090 ppm of zinc respectively. Based on these observations, *G. latifolium* is not a good substitute for imported hops. However, *A. indica* and *V. amygdalina* can substitute hops in beer production. Zinc is an essential trace element present in every cell of the human body. It is an important mineral that makes the immune system work properly and it is also involved in cell growth, cell division, wound healing and breakdown of carbohydrates (Aschner, 2010)[34]. Zinc plays an important role in fermentation and has a positive action on protein synthesis and yeast growth. It also impacts flocculation and stabilizes foam, i.e. promotes lacing.

As expected, mercury was virtually absent in most of the samples, except *A. indica* and *V. amygdalina.* It occurred too high in *A. indica.* This observation casts some doubt on the use of *A. indica* as a substitute since mercury is a highly toxic metal. The high concentration of these heavy metals (cobalt and mercury) in *A. indica* may be due to the environment of growth such as refuse dumps. Thus, *G. latifolium* and *G. kola* could substitute hops in beer production.

Iron was low in all except in *G. latifolium*, Table 5. It was exceptionally low in *V. amygdalina.* Iron is the most important mineral in the human body. Based on this, any of the samples can substitute the other. Iron helps in the formation of heamoglobin and myoglobin (oxygen carrying protein), which is found in red blood cells and muscles respectively. Besides this, it is also a part of many proteins in the body. However, iron in large amounts can give a metallic taste to beer. Iron salts have a negative action at concentrations above 3.2mg/L during wort production, preventing complete saccharification, resulting in turbid worts, and hampering yeast activity (Moll, 1979)[35]. The observation of this author casts some doubt on the use of *G. latifolium* as a substitute for hops since this vegetable contains as high as 8.614mg/L of this mineral.

RANKING

Isomerized hop

The p-value of the test is 0.878 (Table 6) which is higher than 0.05. We conclude that there is insignificant difference among the samples.

Table 6: ANOVA for overall comparison of Isomerized hop and the Nigerian plants

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	458.306	4	114.576	0.297	0.878
Within Groups	17343.520	45	385.412		
Total	17801.825	49			

The output/result of the Post Hoc Test on the average shows that *G. latifolium* has the significance value of 0.919 followed by *G. kola* with 0.819 which implies that *G. latifolium* is the closest substitute to isomerized hop, followed by *G. kola. A. indica* and *V. amygdalina* have significance values of 0.712 and 0.517 respectively which are less than 0.878 but higher than 0.05. This shows that all the Nigerian plants are not significantly different from isomerized hop. Hence, on the average, the closest substitute to isomerized hop is *G. latifolium* and the order of closeness to isomerized hop among the Nigerian plants is *G. latifolium* > *G. kola* > *A. indica* > *V. amygdalina*.

Hop leaf

The p-value of this test is 0.755 (Table 7) which is greater than 0.05 and then, we conclude that there is insignificant difference among all the Nigerian plants (*G. kola, A. indica, V. amygdalina* and *G. latifolium*).

Table 7: ANOVA for overall comparison of Hop leaf and the Nigerian plants

	Sum of squares	**df**	**Mean square**	**F**	**Sig.**
Between Groups	611.877	4	152.969	0.474	0.755
Within Groups	14536.028	45	323.023		
Total	15147.905	49			

The output of multiple comparison using Post Hoc Test (Least Significant Difference) shows that *V. amygdalina* has the highest significance value of 0.964 followed by *G. kola* that has a significance value of 0.679 while those of *G. latifolium* and *A. indica* are 0.439 and 0.288 respectively. None of these values is less than 0.05 which implies that all the tropical plants are not significantly different from hop leaf. Hence, on the average, *V. amygdalina* is the closest Nigerian plant to hop leaf since it has the highest significant value among all the plants investigated. Therefore, the order of closeness to hop leaf is *V. amygdalina* > *G. kola* > *G. latifolium* > *A. indica*.

CONCLUSION

This study has shown that the extracts from tested Nigerian plants could be used as suitable substitutes for hops in beer brewing. Extract of *G. latfolium* had the greatest potential as substitute for isomerized hop extract and that of *V. amygdalina* was the closest substitute for hop leaf extract. Consequently, academic activity in the area of mixtures/blends of extract of plant species which mimic hop taste is strongly recommended.

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