Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/2675
DOI URL: http://dx.doi.org/10.21474/IJAR01/2675
ISSN NO. 2320-5407

*RESEARCH ARTICLE*

# SPECTROSCOPIC METHODS FOR EVALUATION OF HOP EXTRACTS AND EXTRACT FROM GONGRONEMA LATIFOLIUM AS SUBSTITUTE IN THE NIGERIAN BEER INDUSTRY.

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## *Manuscript Info*

***Manuscript History***

Received: 31 October 2016
Final Accepted: 01 December 2016
Published: December 2016

***Key words:-***
Hops, Gongronema latifolium, extract, chemical constituent, metal, beer.

## *Abstract*

The potential of *Gongronema latifolium* as substitute for hops in the Nigerian brewing industry in terms of methanolic extracts was evaluated. The relative proportion of each constituent in hop extracts and that of *G. latifolium* were investigated using Gas Chromatography - Mass Spectrometry (GC-MS). Quantitative investigation of ten metals was carried out using Atomic Absorption Spectrophotometry (AAS). The GCMS results showed that *G. latifolium* contained constituents comparable to those of hops, although some constituents [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 *G. latifolium*. Isomerized hop, hop leaf and *G. latifolium* contained a total number of 14, 11, 10 constituents respectively. The AAS results showed that the concentration of metals investigated in all the samples were calcium (16.300–18.400ppm), sodium (92.019–98.245ppm), potassium (8.297–206.838ppm), magnesium (19.331-22.188ppm), lead (Not detected), manganese (0.426-38.628ppm), cobalt (0.004–0.012ppm), zinc (0.963-17.944ppm), mercury (Not detected) and iron (0.547-8.614ppm). These results indicate that there are minor differences in the chemical constitution of hop extracts and extracts from *G. latifolium* while there is an insignificant difference among the extracts with respect to metal concentration. Hence the result of the analyses presented extract from *G. latifolium* as suitable substitute for hops in the Nigerian beer industry. Consequently, academic activity in the area of mixing/blending of this extract with hop extracts which mimic very closely hop taste is strongly recommended.

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## Introduction:-

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). Hop extracts give beer its bitter taste, improve foam stability, enhance aroma and flavour, and act as antiseptic towards microorganisms (Ashurst, 1971).

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Hops are grown throughout the temperate regions of the world to meet the demands of the brewing industries (Hough *et al*., 1982). 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.

The current economic recession portends that urgent steps should be taken to discourage importation of raw materials in the production and manufacturing sectors of the Nigerian economy. The Nigerian beer industry should not be an exception and therefore the substitution of hops with local raw materials should be of great concern to the research industry.

This piece of work was designed to identify the chemical constituents of imported hops and investigate quantitatively some mineral (metal) contents of hops as well as contrast to *Gongronema latifolium*. It was also designed to find the possibility of this plant serving as substitute for hops in beer production. This level of raw material freedom confers definite economic advantages to the Nigerian brewing industry.

Gongronema latifolium is widely consumed as vegetable in Nigeria. One thing it has in common with hop is that it is bitter like hop but thrives in tropical regions, unlike hop (Ajebesone and Aina, 2004).

## Materials and Methods:-

**Procurement of Materials:-**

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

**Sample Preparation:-**

Except for the isomerised 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 methanolic extracts by steeping procedure.

**GC/MS Analysis:-**

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

**Deconvolution of Chemical Constituents:-**

GC-TOFMS is a benchmark approach for metabolomics data acquisition (Fernie and Shauer, 2008) from chromatographic peaks. 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 constituent chromatograms. In addition, the MS component displays capacity to analyse each eluted chromatographic peak and subject the mass spectra to comparative analysis using a well appointed constituent library of simulated mass spectral information (Finar, 1975; Christian, 2004). In the present investigation, a scanning mass spectrometer was used to obtain chromatograms for the samples. Spectrum matching is achieved by programming the soft ware to compare the chromatogram of the mass spectra to simulated library peaks.

**AAS Analysis:-**

The method as described by Okafor *et al.* was adopted. 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 particleless. 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 analysed 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.

**Constituents Comparison of the Extracts:-**
It is evident from Table 1 that 4,4-dimethyl-2-buten-4-olide; 1,2-dimethyl cyclopropane carboxylic acid; 2,5-dimethyl-2-hexanol; 4,4,5,5-tetramethyl bicyclo hexyl-6-ene-2,3,-dione; 1,2-benzen dicarboxylic acid bis (2-ethyl hexyl) esterand dehydro-cohumulunic acid are present in isomerized hop extract only.
It is also observed that hop leaf extract only contained lupulone, a β-acid known as beta-lupulic acid and octadecanoic acid oxiranyl methylester. All the extracts contained hexadecanoic acid, octadecenoic acid methyl ester, octadecanoic acid methyl ester and 6-octadecenoic acid, 9, 12-octadecadienoic acid and octadecanoic acid, 2-hydroxyl-1, 3-propandiyl ester in common. These results are in agreement with those of Okafor *et al.* (2016) in their characterization of hop extracts and extracts from four selected Nigerian plants by GC-MS and brewing qualities analyses.

However, there are constituents which were present in *G. latifolium* that were absent in imported hops such as octadecanoic acid -2-(2-hydroxyethoxy) ethylester and 9, 12-octadecadiene-1-ol. These observations are consistence with those of Okafor *et al.* (2016). This major differences and minor similarities in the constitution of chemical constituents in *G. latifolium* and those of imported hops is in agreement with the observation of Shellie *et al*. (2009), in their varietal characterization of hop by GC-MS analysis of hop cone extracts and may explain the reason why the organoleptic character of beers brewed with imported hops and that of beers brewed with *G. latifolium* by Okafor and Anichie (1983) were more pronounced while their chemical properties did not differ much.
**Table 1:-** Constituents comparison of all the Extracts

| S/N | Constituet | Isomerized hop | Hop leaf | *G.latifolium* |
|---|---|---|---|---|
| | | Relative Proportion (%) | | |
| 1. | 4,4-dimethyl-2-buten-4-olide | 3.62 | - | - |
| 2. | 1,2-dimethyl-cyclopropane carboxylic acid | 9.90 | - | - |
| 3. | 2,5-dimethyl-2-hexanol | 2.68 | - | - |
| 4. | Dehydro-cohumulunic acid | 5.33 | - | - |
| 5. | 4,4,5,5,tetramethyl-bicyclo hexyl-6-ene-2,3-dione | 9.25 | - | - |
| 6. | 1,2-benzenedicarboxylic, bis (-2-ethylhexyl) ester | 1.14 | - | - |
| 7. | Hexadecanoic acid | 7.84 | 9.54 | 10.69 |
| 8. | Octadecenoic acid, methyl ester | 3.69 | 3.14 | 0.77 |
| 9. | Octadecanoic acid, methyl ester | 1.21 | 2.45 | 0.65 |
| 10. | 6-octadecenoic acid | 28.96 | 43.55 | 44.61 |
| 11. | Octadecanoic acid | 17.92 | 25.56 | - |
| 12. | Hexadecanoic acid, 2-hydroxy -1,3-propanediyl ester | 1.24 | - | - |
| 13. | 9,12-octadecadienoic acid (grape seed oil) | 4.65 | 1.26 | 7.95 |
| 14. | Octadecanoic acid, 2-hydroxyl -1,3-propanediyl ester | 2.57 | 1.63 | 0.61 |
| 15. | Hexadecanoic acid, methyl ester | - | 1.13 | - |
| 16. | Lupulon (beta-lupulic acid) | - | 2.02 | - |
| 17. | Octadecanoic acid, oxiranyl methyl ester | - | 4.13 | - |
| 18. | 9-hexadecenal | - | 5.59 | 1.46 |
| 19 | Octadecanoic acid, 2(-2-hydroxyethoxy) ethylester | | | |
| | 9,12-octadecadien-1-ol | | - | 25.46 |
| 20 | Benzoic acid, 2(aminocarbonyl) | - | | |
| 21 | Hexadecanoic acid-2,3-dihydroxypropyl ester | - | - | 4.91 |
| | | - | - | 2.89 |

Furthermore, another interesting observation is that the relative proportion of chemical constituents which were commonly present in all the extracts are comparatively similar, example, the relative proportion of 6-octadecenoic acid is highest in each extract.

**Table 2:**- Metal Content of the Extracts (ppm).

| Sample | Ca | Na | K | Mg | Pb | Mn | Co | Zn | Hg | Fe |
|---|---|---|---|---|---|---|---|---|---|---|
| Isomerized hop | 16.300 | 98.245 | 206.838 | 19.331 | 0.000 | 0.426 | 0.012 | 0.963 | 0.000 | 0.547 |
| Hop leaf | 17.800 | 92.019 | 8.297 | 21.113 | 0.000 | 0.850 | 0.008 | 1.985 | 0.000 | 0.815 |
| *G .latifolium* | 18.400 | 95.882 | 206.838 | 22.188 | 0.000 | 38.628 | 0.004 | 17.944 | 0.000 | 8.614 |

## Results of Mineral Content of the Extracts:-

The results of the AAS analysis are presented in Table 2. It is evident from Table 2 that calcium is available in all the samples. 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). 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 especially isomerized hop and*G. latifolium*. Sodium plays a major role in controlling blood pressure and blood volume, for proper functioning of muscles and nerves. However, Ted Goldamer had reported that 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). In the presence of sulphate, sodium creates an unpleasant harshness.The concentration of potassium in isomerized hop and *G.latifolium* was comparatively close and therefore *G. latifolium* could substitute isomerized hop. 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).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).

From Table 2, magnesium occurred comparably in all the samples. Thus, each can substitute the other in beer production. 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). Calcium and magnesium chlorides give body, palate fullness, and soft sweet flavour to beer.

From this work, lead is absent in all the samples as expected. Thus, each can substitute the other. Laed is a highly toxic metal that 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, behavioural abnormalities, hearing difficulties and cognitive functions in humans (Donaldin *et al*, 2008).

Table 2 shows that manganese occurred comparatively well in imported hops. In *G. latifolium*, it occurred with some prominence. This observation casts some doubt in the use of *G. latifolium* as a substitute for hops. 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).

Cobalt was somewhat low in *G. latifolium* and comparatively similar in concentration in isomerized hop and hop leaf. On the basis of this observation,*G. latifolium* cannot 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 this metal in all the samples differed very well, lowest in Isomerized hop and highest in *G. latifolium.* Based on this observation, *G. latifolium* is not a good substitute for imported hops. 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). 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 (Barmforth, 2006).

As expected, mercury was absent in all the samples. Mercury is a highly toxic heavy metal. Thus, *G. latifolium* could substitute hops in beer production as far as mercury concentration is concerned.

Iron was low in hops but relatively high in *G. latifolium.* 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 1mg/L during wort production, preventing complete saccharification, resulting in turbid worts, and hampering yeast activity (Moll, 1979). 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. These results are not inconsistence with those of Okafor *et al*. in their comparative studies of bitterness, phytochemical and mineral contents of hop extracts and extracts from four selected tropical plants.

## Conclusions:-

This study has shown that the extracts from *G. latfolium* could be presented as suitable substitute for hops in beer brewing. Extract of *G. latfolium* is closer as a substitute to isomerized hop extract than to hop leaf extract. Consequently, academic activities in the area of mixtures of hop extracts and extracts of *G. latfolium* which mimic very closely hop taste is strongly recommended.

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