



## Comparative Evaluation of Phytochemical Constituents, Bitterness Characters and Essential Oil Contents of Extracts from Four Nigerian Plants as Potential Substitutes for Isomerized Hop Extract in Beer Brewing

Vincent Nwalieji Okafor\*<sup>1</sup>, Joseph Jidefor Obodoeze<sup>2</sup>

<sup>1</sup>Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria

<sup>2</sup>Science Laboratory Technology, Federal Polytechnic, Oko, Anambra State, Nigeria

**Abstract** Four selected Nigerian plants namely *Azadirachta indica* (neem), *Garcinia kola* (bitter cola), *Gongronema latifolium* (heckel) and *Vernonia amygdalina* (bitter leaf) as potential substitutes for hops in beer brewing were evaluated in terms of methanolic extracts. Phytochemical screening of the extracts was carried out using standard methods. The iso-alpha acid and essential oil contents of the extracts were investigated using UV-visible spectrophotometer. Phytochemical results showed that alkaloid content in all the extracts ranged between 3.2-4.8%; tannin ranged from 2.0-4.8%; saponin ranged from 0.80-5.20%; haemagglutini ranged from 3.879-7.240%. The concentration of iso-alpha acid ranged from 7.95-12.53ppm and essential oil content ranged from 0.544g/100g to 1.012g/100g. Consequently, the extracts from tested Nigerian plants could be used as suitable substitutes for hops in beer brewing.

**Keywords** hops, extract, phytochemicals, tropical plants, iso-alpha acid, essential oil

### Introduction

Plants are important to our everyday life. They provide us with food, produce oxygen that we breath and serve as raw material for many industrial products such as clothing, foot wears, building materials and in the manufacture of bio-fuels, dyes, perfumes, pesticides, drugs, beverages and preservatives.

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 [1]. Hop plants are vital to the brewing industry and some of their unique chemicals have the potential to be used in the nutraceutical industry [2]. Hop extracts give beer its bitter taste, improve foam stability, enhance aroma and flavour and act as antiseptic towards microorganisms [3]. Hop plants are grown throughout the temperate regions of the world. Nigeria is in tropical region and since beer production in Nigeria has never declined with ready market as consumption rate continues to increase, the importation of hops becomes inevitable.

The bitterness in beer is caused mainly by iso-alpha acids of hops [1] and marginally by phytochemicals such as tannins and alkalioids. 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 [4]. Phytochemicals are present in hops as well as in many Nigerian plants. This piece of work was designed to investigate quantitatively some



phytochemicals, bitterness and essential oil constituents in isomerized hop extract and contrast them to four selected Nigerian plants.

*Humulus lupulus* L. (hop) is a perennial dioecious climbing plant of hemp (*cannabis*) family and belonging to the order (*urticales*) which also includes the nettle family [5]. Only two species of *Humulus* are recognized: *Humulus lupulus* L. (*H. americanus*, *H. neomexicanus* and *H. cordifolius*) and *H. japonicus* 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 *Cannabaceae* 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 [6].

*Garcinia kola* (bitter cola), an angiospermae, belonging to the family *Guttiferae*, is known in commerce as bitter cola. Bitter cola is a highly valued ingredient in African ethno-medicine because of its varied and numerous uses which are social and medicinal; thus making the plant an essential ingredient in folk medicine, and medicine plants such as *G. kola* are found to be an important source of new chemical substances with potential therapeutic benefits [4, 7].

*Azadirachta* (neem) is a genus of two species of trees in the Mahogany family, *Meliaceae*. Numerous species have been proposed for the genus but only two are currently recognized, *Azadirachta excelsa* and the more economically important tree, *Azadirachta indica* which is the only species in Nigeria [8]. Products made from neem are found to be antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative [9-10].

*Vernonia amygdalina* (bitter leaf) is a shrub or small tree with petiolate leaf of about 6mm in diameter and elliptical shape. The leaves are green with a characteristic odour and a bitter taste [11]. They are used as vegetable and to stimulate the digestive system, as well as reduce fever [12] and as local medium against leech which transmits bilharziasis [13].

*Gonogronema latifolium* (heckle) is a climbing shrub of the family *Asclepiadeceae*. It is known as *utazi* in many Nigerian languages. It grows up to 5m long, stems hollow, all parts soft-hairy to glabrous, with woody base and fleshy roots, containing latex. Leaves opposite, simple and entire; petiole up to 2.5 -3cm long. Fruit, a pair of pendent follicles, each one narrowly cylindrical, 7 -10cm x 1 -1.5cm, yellow and many seeded [8]. Different leaf extracts showed moderate to promising antioxidant, anti-inflammatory, hepatoprotective, anti-plasmodial, anti-asthmatic, anti-sickling, anti-ulcer, analgesic, antipyretic, gastrointestinal relaxing, laxative and stomachic activities [14-18].

In Nigeria, hops are imported, and since beer production in Nigeria has increased recently due to ready markets, the importation of hops becomes inevitable. Thus, huge amounts of foreign exchange are being spent by this sector in importation, hence, the urgent need to investigate some potential Nigerian plants that can substitute hops in the Nigerian beer industry. This study takes into consideration other competitive uses of these selected Nigerian plants.

## Methods

### Phytochemicals

Phytochemicals analyzed include: alkaloids, tannins, saponins and hemagglutinins. All these were determined based on methods of analyses described by AOAC (1980) [19] as adopted by other researchers [20-24].

### Iso-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. Concentration of the iso-alpha acid was calculated as  $73.79A_{325} - 51.56A_{355} - 19.07A_{275}$  mg/l where A = absorbance at the specified wavelength according to Hough *et al.*, 1982 [1].

### Essential Oil Determination

Steam distillation method as adopted by Adama *et al.* (2011) [25] was employed.



## Results and Discussion

### Phytochemicals

The alkaloid contents of the samples studied are shown in Table 1. 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. 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 [26-28].

Tannin was present in all the samples but highest in *V. amygdalina* with 4.8% and lowest in *G. kola* with 2.8%. Table 2 shows tannin content is somewhat comparatively uniform in all the samples except in *G. kola* and thus all the local vegetables except *G. kola* could substitute hops. Tannins (commonly referred to as tannic acids) are polyphenols present in many plant foods that form colloidal solution in water [9]. 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 [29] 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 [30].

**Table 1:** Alkaloid content in the samples

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

**Table 2:** Tannin content in the samples

Sample	Tannin (%)		
	Mean	St Dev	Range
Isomerized hop	3.6	0.300	0.6
<i>G. kola</i>	2.8	0.265	0.5
<i>A. indica</i>	4.0	0.200	0.4
<i>V. amygdalina</i>	4.8	0.458	0.9
<i>G. latifolium</i>	4.4	0.361	0.7

**Table 3:** Saponin content in the samples

Sample	Saponin (%)		
	Mean	St Dev	Range
Isomerized hop	2.8	0.458	0.9
<i>G. kola</i>	1.2	0.265	0.5
<i>A. indica</i>	5.2	0.436	0.8
<i>V. amygdalina</i>	0.8	0.173	0.3
<i>G. latifolium</i>	2.4	0.500	1.0



**Table 4:** Concentration of Haemagglutinin in the samples

Sample	Concentration (mg/g)		
	Mean	St Dev	Range
Isomerized hop	6.372	1.407	2.777
<i>G.kola</i>	3.879	0.875	1.724
<i>A. indica</i>	7.270	0.465	0.819
<i>V. amygdalina</i>	6.654	0.399	0.766
<i>G. latifolium</i>	6.672	0.809	1.547

**Table 5:** Iso-alpha Acid of the Extracts

Extract	Iso-alpha acid (ppm)
Isomerized hop	12.53
<i>G. kola</i>	8.78
<i>A. indica</i>	10.12
<i>V. amygdalina</i>	9.44
<i>G. latifolium</i>	9.67

**Table 6:** Essential Oil of the Extracts

Extract	Essential oil (g/100g)
<i>G. kola</i>	0.878
<i>A. indica</i>	1.012
<i>V. amygdalina</i>	0.544
<i>G. latifolium</i>	0.967

Except *A. indica* that contained the highest saponin content of 5.2%, isomerized hop, and *G. latifolium* were comparable in saponin contents. *V. amygdalina* had the lowest, followed by *G. kola* (Table 3). These factors showed that *G. latifolium* could substitute imported hops. 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 [31].

Except in *G. kola* and *A. indica* where the concentration of haemagglutinin was as low as 3.879mg/g and as high as 7.270mg/g respectively, all the other samples were virtually in the same range. Therefore, except *G. kola*, all the others could substitute one another in beer brewing. This evidence is clearly shown in Table 4. Haemagglutinin refers to a substance that causes red blood cells to agglutinate, a process known as haemagglutination. Antibodies [32] and lectin [33] are common known haemagglutinins.

#### 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 *G. kola*, the lowest iso- alpha acid of 8.78ppm. Table 5 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) [25] in their investigation of bitter leaf as local substitute for hops in the Nigerian brewing industry. These results are consistent with the report of Ashurst (1971) [34] 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.

#### Essential Oil

From Table 6, it was observed that the results obtained for essential oil content in all the extracts of the Nigerian plants were virtually in the same range but especially lowest in *A. amygdalina* and comparably. The essential oil content of the Nigerian plants except *A. amygdalina* fell within the range of hop oil content of 0.88-1.63 g/100g as reported by Hough *et al.* Hough *et al* had reported that fresh hop contained 1.63g oil/100g which fell during storage



to 0.88g/100g [1]. The low content of oil in *A. amygdalina* is attributed to the absence of the metabolite, 9,12-octadecadienoic acid- the grape seed oil in this plant species as reported by Okafor *et al.*, 2016 [23].

### Conclusion

This study has shown that phytochemicals (alkaloids, tannins, saponins and haemagglutinins), iso-alpha acids and essential oil contents investigated in the Nigerian plants are comparatively similar with those of the isomerized hop extract. Consequently, these Nigerian plants could be used as possible substitutes for isomerized hop extract in the Nigerian beer industry.

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