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Phytochemical Constituents of Extracts of Hops and Some Potential Nigerian Hop Substitutes: A Comparative Study in Beer Brewing

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Abstract

Phytochemical constituents of methanolic extracts from four Nigerian bitter vegetables namely *Garcinia kola* (bitter cola), *Azadirachta indica* (neem), *Vernonia amygdalina* (bitter leaf) and *Gongronema latifolium* (heckel) and in comparison with hop extracts were evaluated quantitatively. Extracts of the plant parts commonly consumed by people were used in this study. The aim was to ascertain the potentiality of using the Nigerian vegetables as suitable and available hop substitutes in beer brewing with respect to quantitative constitution of various phytochemicals in the extracts of these vegetables and the hops. Analyses of alkaloids, tannins, saponins, oxalates, phytatic acid, trypsin inhibitors, cardiac glycosides, haemagglutinins, cyanogenic glycosides and hydrogen cyanide were carried out using their respective standard methods. The phytochemical constituents of the vegetables were statistically ranked by the application of Analysis of Variance (ANOVA). The results showed that all the phytochemicals analyzed were present in all the plant extracts. Alkaloid content in all the extracts ranged between 3.2 and 4.8%, tannin ranged from 2.0 to 4.8%, saponin (0.80-5.20%), phytate (0.99-1.68%), trypsin inhibitors (2.80-17.30%), hemagglutinin (3.879-7.240%) and cardiac glycoside (3.5-6.0%). The concentration of oxalate ranged from 0.0405 to 0.1020mg/100g, cyanogenic glycoside (0.216-0.810ppm) and hydrogen cyanide (0.540-1.404ppm). It was established from ranking that the order of closeness of the extracts from the bitter vegetables 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 bitter vegetables 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: Phytochemicals, Extracts, Hops, Hop substitutes, Beer brewing

INTRODUCTION

The importance of plants in human existence cannot be overemphasized. They produce oxygen we breath, provide us with life essentials such as food, energy, money and serve as raw materials for many industrial products such as clothes, foot-wears, building materials and are useful in the manufacture of drugs, beverages, biofuels, pesticides, perfumes, preservatives etc. From medieval times, herbs of plants had been used to flavor and preserve fermented malt liquors but only hop is used on commercial scale today. Hops, the female flowers of the hop plant are grown throughout the temperate regions of the world solely to meet the demands of the brewing industry [1].

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, in this case, *Saccharomyces cerevisiae* for top fermenting and *Saccharomyces carisbergensis* or *Saccharomyces uvarum* for bottom fermenting [1]. Essential change here is conversion of glucose to ethanol. The addition of processed hop extract allows some bittering, flavouring and aroma-enhancing powers and foam stability in the beer as small polymeric units. In addition, hops 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 [2]. The small polymeric units that accounts for hop characters in beer originate from hop's phytochemicals.

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 [3]. Examples are alkaloids, tannins, saponins, flavonoids, terpinoids, oxalates, trypsin inhibitors, glycosides etc. Alkaloids are low molecular-weight nitrogen containing compounds and due to the presence of a heterocyclic ring containing a nitrogen atom, are typically alkaline. The presence of alkaloid and other secondary metabolites in plants enhances plant reproductive rates either by improving defenses against biotic and abiotic stress or by affecting pollinators and seed/fruit dispenser visitation. Defensive strategies include predator repellence by toxicity or bitterness taste or change repair by antioxidant system [4, 5]. Tannins (or tannoids) another group of phytochemicals are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids [6]. Another class of phytochemicals, the saponins are glucosides with foaming characteristics that consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C_{27}) or a triterpene (C_{30}) [7]. Oxalates are natural substances and a class of phytochemical found in many foods which bind to calcium during digestion in the stomach and intestines and leave the body in stool. Oxalate that is not bound to calcium travels as a waste product from the blood to the kidneys where it leaves the body in the urine [8]. Phytic acid, also known as inositol hexaphosphate (IP6), or phytate is a powerful antioxidant that helps in ridding the body off heavy metals and other toxins [3]. Trypsin inhibitors are phytochemicals that reduce the availability of biological active trypsin, an enzyme essential to nutrition of many animals, including humans [5]. Haemagglutinin refers to a substance that causes red blood cells to agglutinate, a process known as haemagglutination [4]. Cardiac glycosides are organic compounds containing a glycoside that acts on the contractile force of the cardiac muscle and because of their potency in disrupting the functions of the heart, most are extremely toxic [3]. Cyanoglycosides account for approximately 90% of the wider group plant toxins known as cyanogens [9]. Potential toxicity of cyanoglycosides arises from enzymatic degradation to produce free hydrogen cyanide (cyanogenesis), resulting in acute cyanide poisoning. These glycosides are found as secondary metabolites in several plants [10]. Hydrogen cyanide, sometimes called prussic acid is an inorganic compound which is colourless and extremely poisonous liquid. [11]

The alkaloids and tannins in hops are the source of the bitter tastes while the saponins supply foam to the beer and numerous other phytochemicals are responsible for other properties which the hops supply to the beer. It is the supply of these qualities from hop phytochemicals that distinguishes beers from plant equivalent of hops or hop produce. The comparison of hop phytochemicals with plants' phytochemicals and the substitution of these chemical moieties from plants' phytochemicals that are capable of mimicking hop extracts are the bases of this work.

In Nigeria, hops are imported, and with the expansion of the brewing industry huge amounts of money are spent by this sector for the importation of hops. Studies abound that seek to substitute hops with tropical bitter vegetables [12-15] but little or none has evaluated phytochemical constitutions of the plants and contrast to hops.

A. indica is used in some parts of Nigeria for treatment of malaria while *G. kola* is used in some areas for the treatment of stomach ache and gastritis whereas *G. latifolium* and *V. amygdalina*are widely consumed as food. [16-19]. One thing common to all the four vegetables is that they are bitter like hops but thrive in tropical regions unlike hops [20].

This study therefore is aimed at investigating the suitability of the four Nigerian vegetables as potential hop substitutes in beer brewing with regards to quantitative constitution of various phytochemicals in the vegetables and in comparison with those of imported hops.

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 seeds of *G. kola* and the leaves of *A.indica*, *G. latifolium* and *V. amygdalina* were obtained from the herbarium of Nnamdi Azikiwe University, Awka. Chemicals used were as detailed by

AOAC, ASBC and IOB [21-23] and were of analytical grade. The non-analytical grade chemicals were purified by standard procedure when required.

Sample Preparation and Extraction

The sample preparation and extraction were done as detailed in our previous works [24,25]. Except for the isomerised hop extract prepared by Ritchies, each plant sample was milled and vacuum dried at 50°C. Two hundred grams (200 g) of each plant material thus prepared was stored in a desiccator for the rest of the experiment.

The methanol extract was prepared by steeping 50 g of the dry powdered plant material in 200 cm^3 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°C for 2 hours to obtain each extract.

Phytochemical Screening

All the phytochemicals screened were determined based on methods of analysis described by AOAC (1980) [26] as adopted and described by other researchers [27-33]. These methods had been described in our previous works [13, 14, 24].

RESULTS AND DISCUSSIONS

The results of the phytochemicals in all the extracts are presented in table 1. 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 [34-36].

Tannin was highest in *V. amygdalina* with 4.8% and lowest in Hop leaf with 2.0%. *V. amygalina* and *G. latifolium* contained 4.8% and 4.4% tannin respectively and *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*. Thus, all the local vegetables except *G. kola* could substitute hops, if the volumes of their extracts are somehow reduced during hopping. Tannins (commonly referred to as tannic acids) are polyphenols present in many plant foods that form colloidal solution in water [37]. These solutions have astringent (mouth puckering) taste. Tannins have been reported by Siddiqui and Ali [38] 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 [39].

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*. 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. The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins have a bitter taste and some saponins are toxic and are known as sapotoxin. Saponins have been reported to be helpful in reducing cholesterol during treatment of heart problems, and in building body structure [40].

Closer examination of the Table showed that Oxalate concentration was high in *G. kola* but comparatively close in all the other samples. Therefore, *A. indica*,

Table 1: Physiochemical properties of the Extracts

Extracts	Alkaloid %	Tannin%	Saponin %	Oxalate mg/100g	Phytic acid %	Trypsin inhibitor %	Haemoglobin Mg/g	Cardiac glycoside,%	Cynogenic glycosideppm	Hydrogen cyanide ppm
Isomerized hop	3.2 ± 0.265	3.6 ± 0.300	2.8 ± 0.458	0.0405± 0.009	1.39 ± 0.0173	6.45± 0.427	6.372± 1.407	4.5 ± 0.917	0.648± 0.0451	0.756 ± 0.0674
Hop leaf	4.0 ± 0.300	2.0 ± 0.173	0.3 ± 0.346	0.0432 ± 0.00735	1.68± 0.0624	7.60± 0.0361	6.669± 0.584	3.5± 0.624	0.810± 0.1000	0.648 ± 0.1046
G.kola	4.0 ± 0.436	2.8 ± 0.265	1.2 ± 0.265	0.10200± 0.00260	0.99 ± 0.0361	2.80± 0.608	3.879± 0.875	4.0± 0.850	0.756 ± 0.0490	0.648 ± 0.1034
A.indica	4.0 ± 1.73	4.0 ± 0.200	5.2 ± 0.436	0.0540 ± 0.00409	1.16 ± 0.0608	9.60± 0.600	7.270± 0.465	6.0± 0.346	0.216 ± 0.0779	0.540 ± 0.1201
V.amygdalina	4.8± 0.346	4.8 ± 0.458	0.80± 0.173	0.0486 ±0.00791	1.62 ± 0.1253	16.45± 1.262	6.650± 0.359	5.0± 0.954	0.594 ± 0.0896	0.756 ± 0.0527
G.latifolium	4.0 ± 0.173	4.4 ± 0.361	0.5± 1.000	0.0540± 0.00368	1.51 ± 0.1820	17.30 ± 2.270	6.672± 0.809	5.5± 0.400	0.702 ± 0.0744	1.404 ± 0.4210

V. amygdalina and *G. latifolium* can substitute hops. Oxalate is a chelating agent for metal cations and thus has the ability to attract calcium cations to form calcium oxalate which causes nephrolithiasis (kidney stone). The toxicity of oxalic acid is due to kidney failure caused by precipitation of solid calcium oxalate [41].

Phytic acid is virtually of the same range in all the samples especially hop leaf, *V. amygdalina* and *G. latifolium.* Percentage content of phytic acid in *G. kola* and *A. indica* did not differ much while isomerized hop was moderate in comparison with the others. Hence, hop leaf, *V. amygdalina* and *G. latifolium* could substitute one another while *G. kola* and *A. indica* as well as isomerized hop could substitute one another. Phytic acid is a chelating agent that binds to minerals, metals or anything else it comes in contact with and takes them out of the body leading to loss of minerals in the body and its resultant consequences [42].

The percentage trypsin inhibitor units were virtually the same in isomerized hop, hop leaf, and *A. indica* but lowest in *G. kola* and especially highest in *V. amydalina* and *G. latifolium*. Based on these observations, *A. indica* can substitute imported hops in beer brewing. However, when the concentrations of *V. amygdalina* and *G. latifolium* are reduced by half and that of *G. kola* increased thrice, then, it will be possible that all the samples could substitute one another. The trypsin inhibitors are reported to be one of the major toxic components of legumes [43].

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. Antibodies and lectin are common known haemagglutinins [44, 45].

From this study, it is evident that cardiac glycosides were available in all the samples. This showed that isomerized hop and *G. kola* can substitute each other while *V. amygdalina* and *G. latifolium* can also substitute each other. When the concentration of Hop leaf and *A. indica* are increased and decreased respectively to a little extent during hopping, then all the Nigerian bitter vegetables could substitute imported hops. Cardiac glycoside content of isomerized hop, hop leaf and *G. kola* were comparatively similar while the cardiac glycoside content of *A. indica*, *V. amygdalina* and *G. latifolium* were also comparatively similar. On the bases of these observations, *G. kola* can substitute imported hops whereas *A. indica*, *V. amygdalina* and *G. latifolium* can substitute one another. Therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure, congestive heart failure, and as heart tonics, diuretics and emetics [8].

The concentration of cyanogenic glycosides or cyanoglycoside was relatively low in *A. indica.* Its concentrations in isomerized hop and *V. amygdalina* did not differ much and were comparatively uniform in those of hop leaf, *G. kola* and *G. latifolium*. Thus, except *G. kola,* all the other samples could substitute one another. It has been established that clinical symptoms of acute cyanide poisoning include rapid respiration; drop in blood pressure, rapid pulse, headache, dizziness, vomiting, diarrhoea, mental confusion, blue discolouration of the skin due to lack of oxygen (cyanosis), twitching and convulsions [46-48]. The presence of cyanogenic glycoside in hops, though in very little amount may explain the reason why people who consume beers in excess sometimes vomit, complain of headache and feel dizzy.

With the exception of *G. latifolium* where the concentration of hydrogen cyanide is especially high, it is evident from this work that *V. amygdalina* is a very good substitute to isomerized hop while *G. kola* is a good substitute to Hop leaf. The concentration of *A. indica* should be increased while that of *G. latifolium* should be reduced by half if two of them are to be used as substitutes. A hydrogen cyanide concentration of 3,500 ppm will kill a human in about 60 seconds [49]. The toxicity is caused by the cyanide ion, which halts cellular respiration by acting as a non-competitive inhibitor for an enzyme in mitochondria called Cytochrome C oxidase [50, 51].

RANKING

Isomerized hop

The p-value of test is 0.633 (Table 2) which is greater than 0.05. Then, there exists enough evidence to accept the null hypothesis and conclude that there is insignificant difference among the samples investigated.

Table 2. ANOVA for comparison of phytochemicals in isomerized hop and the Nigerian bitter vegetables

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	35.028	4	8.757	0.645	0.633
Within Groups	610.824	45	13.574		
Total	645.852	49	13.574		

The result of the multiple comparisons using the post hoc test (Table 3) showed that *A. indica* has the highest significance value of 0.618 which implies that the sample is the closest among all the plants to isomerized hop. Also, the significance value of *G. kola* to isomerized hop is 0.605 which is the second significance value in

ranking among the values. This implies that *G. kola* is also close to isomerized hop but not as close as *A. indica*. Other samples, i.e. *V. amygdalina* and *G. latifolium* have significance values less than 0.605 but higher than 0.05 which shows that the samples are not significantly different from isomerized hop.

Table 3: Post hoc tests for comparison of isomerized hop extract and those of Nigerian plants

(+A) Factor	(*J) Factors	Mean Difference (A-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	C	.85815	1.64766	.605	-24604	4.1767
	D	-.82790	1.64766	.618	-4.1465	2.4907
	E	-1.17661	1.64766	.479	-4.4952	2.1419
	F	-1.41855	1.64766	.394	-4.7371	1.9000

+A = Izomerized hop extract

*J (C-F) = Nigerian plants

Hop leaf

The p-value of the test is 0.645 which is greater than 0.05 (Table 4) and then, we have enough evidence to conclude that there is insignificant difference among the samples of interest (hop leaf, *G. kola*, *A. indica, V. amygdalina* and *G. latifolium*).

Table 4. ANOVA for comparison of phytochemicals in Hop leaf and the Nigerian bitter vegetables

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	34.636	4	8.659	0.628	0.645
Within Groups	620.672	45	13.793		
Total	5.308	49			

The multiple comparisons using post hoc test (Table 5) showed that *A. indica* has the highest significance value of 0.637 which implies that this plant is closest among all to hop leaf (control). Other extracts, i.e., *G. kola*, *V. amygdalina*, and *G. latifolium* have significance values less than 0.637 but higher than 0.05. This shows that the plants are not significantly different from hop leaf.

Table 5: Post hoc tests for comparison of hop leaf extract and those of Nigerian plants

(+B) Factor	(*J) Factors	Mean Difference (B-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	C	.89752	1.66089	.592	-2.4477	4.2427
	D	-7.8853	1.66089	.637	-4.1337	2.5667
	E	-1.13724	1.66089	.497	-4.4824	2.2080
	F	-1.37918	1.66089	.411	-4.7244	1.9660

+B = Hop leaf extract

*J (C-F) = Nigerian plants

CONCLUSION

Phytochemical assay showed that there is no significant difference in percentage alkaloid, tannin, saponin, phytate, trypsin inhibitor and cardiac glycoside; and concentrations of oxalate, heamagglutinin, cyanoglycoside and hydrogen cyanide between the control and the Nigerian plants.

This study has shown 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. latfolium* having the greatest potential as substitute for isomerized hop extract and that of *V. amygdalina*, the closest substitute for hop leaf extract.

AUTHOR'S 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 and as well supervised authors RIA and UWO during laboratory work. Author CPO coordinated the laboratory analyses, author JNO assisted in the procurement of research materials and literature on beer while author MCO provided some literature on phytochemicals. All authors read and approved the final manuscript.

CONFLICTING INTERESTS

Authors declare that they have no conflicting interests.

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