CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

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 clothings, 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. The hop plant is grown in the temperate regions of the world, solely to meet the demands of the brewing industry (Hough, *et al*., 1982).

Hop extracts contribute to beer foam stability and also provide hop flavour, hop character and preservative properties to the beer (Laws, 1981).

The Webster's Dictionary defines beer as an alcoholic drink made from yeast fermented malt, flavoured with hops.

Beer production worldwide is a viable industry. Among commercial beverages in 2006, beer ranks fourth in per capita consumption behind carbonated soft drinks, bottled water and coffee followed by milk and fruit drinks in the United States of America. Per capita beer consumption rose rapidly during the second world-war, declined during the 1950s and early 1960s, increased before peaking in the early 1980s and has generally leveled-off thereafter (Goldamer, 2008).

A similar trend is reported of the beer industry in Nigeria by Badmus (2013) who observed that the Nigerian beer industry is a very vital component of Nigeria's non-oil sector and has largely contributed to economic growth in recent times. This can be attributed to the country's favourable demographics with populous and vibrant youth and growing middle class. This, along with a growing, largely youth population with increased disposable incomes is the constant drive that increased beer consumption in Nigeria.

Even as Western beer consumption slows down due to the global economic downturn, Nigeria's beer industry continues to thrive. The country has the second largest beer market in Africa, after South Africa and with the largest population in Africa, a growing middle class and a large number of drinking beer age, the brewing multinationals are struggling for a position in a market that shows plenty of room for expansion. Indeed, beer drinking has been steadily increasing in recent decades even in countries where alcoholic beverages are not traditional. Hence, beer has become an international drink, especially among young people (Svenden and Lund, 2000).

Despite the fact that Islamic Sharia law bans the sale and consumption of alcohol in some of Nigeria's Northern states, consumers continue to find means of buying beer. Alcohol in the Northern States is sold in Military facilities, which are federal territory and thus not subject to state laws (Badmus, 2013).

Beer production in Nigeria has increased recently due to ready markets. This assertion is expressed by the annual consumption rate of beer in Nigeria as shown in Table 1.1. Hence, the importation of hops to meet the demand of the brewing industries continues to constitute a significant proportion of the Nigerian economy.

Table 1.1: Annual Consumption rate of beer in Nigeria from 2008 to 2011

	Quantity Consumed (mn hl)*
2008	115
2009	126
2010	151.2
2011	151.5

(Source: NIBREWNEWS, 2012). *million hectolitre

Some pioneer work showed that leaves of the vegetable, *Gongronema latifolium* (*utazi*) have great potential as substitute for hops in tropical beer brewing. It was found out that this plant possessed some antiseptic properties against beer spoilage microrganisms. The chemical properties of beer brewed using this plant did not differ much from that brewed with hops though their organoleptic differences were pronounced (Okafor and Anichie, 1983). The authors however did not characterize the vegetables as they only used it for brewing and sensory analysis.

Ajebesone and Aina (2004) characterized four bitter plants used for food in Africa viz: *Azadirachta indica* (neem), *Garcinia kola* (bitter kola), *Gongronema latifolium* (heckel) and *Vernonia amygdalina* (bitter leaf).They concluded that

these Nigerian bitter vegetables can serve as substitutes for hops in tropical beer brewing, but they only carried out proximate analysis of the vegetables.

Azadirachta indica is used in some parts of Nigeria for treatment of malaria while *Garcinia kola* is used in some areas for the treatment of stomach ache and gastritis whereas *Vernonia amygdalina* and *Gongronema latifolium* are widely consumed as vegetables. One thing common to all the four plants is that they are bitter, like hops, but thrive in tropical regions, unlike hops (Ajebesone and Aina, 2004).

This piece of work was designed to identify the chemical constituents of imported hops, investigate quantitatively some nutritional and anti-nutritional values of imported hops and study the mineral (metal) contents of hops as well as contrast to some Nigerian plants. It was also designed to find the possibility of these plants serving as substitutes for hops in beer production.

If it is categorically established in this study that these Nigerian plants can substitute hops in the production of premium quality and world class beers, the brewing industries in Nigeria will no longer depend on imported hops. This level of raw material freedom confers definite economic advantages to the Nigerian brewing industry.

1.2 HISTORICAL PERSPECTIVE

1.2.1 History of Beer

The word beer is derived from the latin word *bibere* which means to drink (Okafor, 2007). 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 (Micheal, 2004). The invention of bread and beer has been argued to be responsible for man's ability to develop technology and build civilization (Nelson, 2005). 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 (Nelson, 2005).

The U.S. beer industry started in the 1840s and 1850s with the introduction of lager style beers, brought by German immigrants (Arnold, 2005). 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 ninetieth century, there were almost 1,300 breweries (Elzinga, 2005).

1.2.2 The Nigerian Beer Industries

In Nigeria, the Nigerian Breweries Plc, incorporated in 1946, is the pioneer and largest brewing company. Its first bottle of beer, Star lager rolled off the bottling lines of its Lagos brewery in June 1949 (Nigerian Stock Exchange, 2012).

The Nigerian Breweries Plc audited results, according to NIBREW NEWS 2013, amongst other issues reported the following:

"That apart from its Lagos brewery, other breweries were commissioned by the company, including Aba brewery in 1957, Kaduna brewery in 1963, and Ibadan brewery in 1982. In September 1993 the company acquired its fifth brewery in Enugu state, and in October 2003, its sixth brewery, sited at Ama in Enugu. Ama brewery is the largest brewery in Nigeria and one of the most modern worldwide. In October 2011, Nigerian Breweries acquired majority equity interests in Sona Systems Associated Business Management Limited (Sona Systems) and Life Breweries limited from Heineken. Sona System's two breweries in Ota and Kaduna, and Life Breweries in Onitsha have now become part of Nigerian Breweries Plc, together with three brands, Goldberg lager, Malta gold and Life continental lager. Nigerian Breweries Plc now has eight operational breweries from which its products are distributed to all parts of Nigeria, in addition to the Maltina plants in Aba and Kaduna.

The company has a portfolio of high quality brands including Star lager (launched in 1949), Gulder lager (1970), Legend extra stout (1992), Heinekenn lager (1998), Goldberg lager (2011) and Life continental lager (2011)".

Guinness Nigeria, a subsidiary of Diageo Plc of the United Kingdom, was incorporated in 1962 with the building of a brewery in Ikeja, Lagos state. The brewery was the first outside of Ireland and Great Britain. Other breweries have been opened overtime – Benin City brewery in 1974, Ogba brewery in 1982 and Port-Harcourt brewery in August 2013. Guinness Nigeria Produces the following beer brands – Foreign extra stout (1962), Harp lager beer (1974), Guinness extra smooth (2005), Satzenbrau (2006), Dubic extra lager (2012), Smirnoff ice (2006), SNAPP (2012), and Orijin (2013) (Badmus, 2013).

From the foregoing, it is evident that Nigeria has moved from a duopoly beer industry to an oligopoly one. Heineken has 71% share, through its two subsidiaries, Nigerian Breweries Plc with 61% market share and Consolidated Breweries with a 10% market share. Diegeo has a 27% market share through its stake in Guinness Nigeria. South African Breweries Miller (SABM) is a more recent entrant to the market and has a growing but very significant stake in the industry. NB Plc has the largest capacity coverage with about 8 breweries located across the country (estimated to have a total annual capacity of 13 million hectoliter {mn.hl}). Guinness operates four breweries (total annual of 7.5 mn.hl. by 2014 due to on-going capacity expansion), SABM has built up its capacity (by

acquisition) to approximately 1.8 mn.hl, which includes Pabod breweries in Port Harcourt, International breweries in Ilesa and Onitsha (Badmus, 2013).

SABM has grown from regional player into the world's second largest brewer by volume in the space of 15 years and with two brands, Hero and Trophy lager beers, the brewery giant is gradually winning the hearts of loyalists of the established brands. While Hero lager beer stems from Onitsha, the eastern part of Nigeria; Trophy, which was originally launched by International breweries Plc, Ilesa, Osun state in 1978 was taken over in 2011 by the Management of SAB Miller. These two brands have remained remarkable choices of lager with strong and notable presence in Eastern and South Western Nigeria respectively.

The beer industry in Nigeria grew in value by 2.18% in 2009 making it worth USD 2.7 billion and since Nigerians consume just 11 litres of beer per head of population per day, the market has plenty of room to continue expanding (Badmus, 2013). Drinking alcohol is a social activity in Nigeria, and therefore beer controls 80% of the country's alcoholic sales. Thus, beer is the most popular alcoholic drink in the country, making up more than 80% of all alcoholic sales (Badmus, 2013).

1.3 HYPOTHESIS OF STUDY

The hypothesis of study is the scientific bases which controls the fundamental relationships of the individual components that result in a given product. This special case of the 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. 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 are phytochemicals from the hop additive. Usually such chemicals are in classes such as flavones and terpnoids. 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 is the bases of this Dissertation.

1.4 STATEMENT OF PROBLEM

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 the importation of hops.

As a result of the growing trend towards sourcing of local substitutes for industrial raw materials in Nigeria, a lot of efforts have been made in the brewing

industry for the substitution of barely with some local cereals. However, the substitution of hops with local raw materials has not received similar attention.

It is our considered opinion to establish that the four Nigerian plants {*Garcinia kola* (bitter cola), *Azadirachta indica* (neem), *Vernonia amygdalina* (bitter leaf) and *Gongronema latifolium* (heckel)} can substitute hops in the production of premium and world class beer so that brewing industries in Nigeria will no longer depend on imported hops, but have options of local, available and inexpensive substitutes. The economic importance of such substitution is considerable and forms a reseanable boost for research in this area.

1.5 AIM AND OBJECTIVES

The aim of this work was to investigate the potential of four Nigerian plants namely *Garcinia kola, Azadirachta indica, Vernonia amygdalina* and *Gongronema latifolium* as local and available hop substitutes in the production of premium and world class beer.

The objectives of this research were as follows:

(1) To quantitatively establish the chemical constituents in both hops and the Nigerian plants.
(2) To screen quantitatively phytochemicals present in hops and the Nigerian plants.
(3) To quantitatively analyze the hops and the Nigerian plants for metals concentrations.
(4) To quantitatively contrast some physicochemical parameters of beers brewed with hops and those brewed with the plants.
(5) To statistically rank the local plants using imported hops as control.

1.6 JUSTIFICATION OF STUDY

Nigeria was an enthusiastic signatory to the Millennium Development Goals (MDGs) as well as the United Nations Millennium Declaration in 2000. While the MDGs are for all mankind, they centre primarily on children development because children are the most vulnerable when essentials such as food, water and health care are limited. The aim of the MDGs is to encourage development by improving social and economic conditions in the World's poorest countries. The Millennium Declaration produced by the United Nations, asserts that every individual has the right to dignity, freedom and equality, a basic standard of living that includes freedom from hunger and violence and encourages tolerance, and solidarity (UNMD, 2000)

If Nigeria must attain the MDGs' purposes such as eradication of extreme poverty and hunger, then, there is the urgent need to use local raw materials in the production of beer in the country instead of depending solely on importation of raw materials. The benefits when achieved include *viz*:

(a) There will be employment opportunities for the citizenry since majority of Nigerians at present are either unemployed or underemployed.

(b) Foreign exchange will be conserved.

(c) Nigerian brewing industries will no longer depend on the importation of hops, thereby encouraging mass production of the plants solely to meet the demands of already expanded brewing industries in Nigeria.

(d) There will be economic agro based jobs for local farmers.

1.7 SCOPE OF STUDY

Five (5) different bitter herbs were used for evaluation. Four (4) were Nigerian and the other was the leaves of the female hop inflorescence plant. Processed female hop inflorescence was also evaluated. The hops were used as control. This gives evaluation of six different samples.

Each Nigerian herb was collected from the gardens of Pope John Paul II Major Seminary, Awka. The herbs were identified by taxonomists in the herbaria of two different institutions namely Department of Botany, Nnamdi Azikiwe University, Awka and Department of Forestry, Anambra State Ministry of Environment, Awka.

Three (3) replicate methanolic extractions of each plant part sample and the isomerized hop inflorescence were prepared. Similarly, 3 ethanolic extractions of the plant samples were prepared. These extractions result in 36 experimental units.

Each extract sample was subjected to replicate Thin Layer Chromatography (TLC) tg give 72 experimental units.

Raw extract of the 36 experimental units were analysed by GC/MS to give 36 experimental units.

The three pooled methanolic extracts were subjected to 10 different tests for phytochemical contents to result in 30 experimental units.

The raw extracts were subjected to AAS analysis for ten different metals to give 360 experimental units.

Each Nigerian plant sample was subjected to nine brewing tests. This gives 36 experimental units for brewing quality test. Female hop leaves were also subjected to 9 brewing tests. Isomerized hop inflorescence prepared by Ritchies, England was also used nine times. Therefore, brewing tests alone gave 54 experimental units.

Quantitative analyses of chemical, phytochemical, metal, physicochemical and spectrophotometric evaluation of all samples were validated by the application of Analysis of Variance (ANOVA).

Therefore, the number of experimental units required to achieve the objectives of this study is 588. When each experimental unit was replicate to two observational units, then the Dissertation requires 1,176 experimental outcomes to satisfy statement of objectives. This does not include the process, time and software necessary for ranking efficiency of each plant.

CHAPTER TWO

LITERATURE REVIEW

2.1 BREWING INGREDIENTS

Beer, a brewed beverage is made principally from malt (partially germinated barley), hop, water and yeast.

2.1.1 Barley

Barley (*Hordeum vulgare L.),* a member of the grass family, is a major cereal grain. In 2007 ranking of cereal crops in the world, barely was fourth both in terms of quantity produced (136 million tons) and in area of cultivation (566,000 square kilometers) (FAOSAT, 2009).

Barley grows in two-row, four-row or six row form as distinguished by the number of seeds on the stalk of the plant. European brewers traditionally use two-row type because it has a better starch/husk ratio and because of its malty flavour. America often preferred the six-row type because of the higher levels of disastatic enzymes and protein which makes it better suited for mashing adjuncts such as corn or rice.

2.1.2 *Humulus lupulus* (Hop)

Hops, a minor 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).

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). 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). 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 (Crombie and Crombie, 1975). 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).

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 (Fig. 2.1) (Burgess, 1964) 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.

The brewing value of the hop is found in its resins and essential oils. Peacock (2009) 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).

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.

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, while 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 (Westwood, 1994).

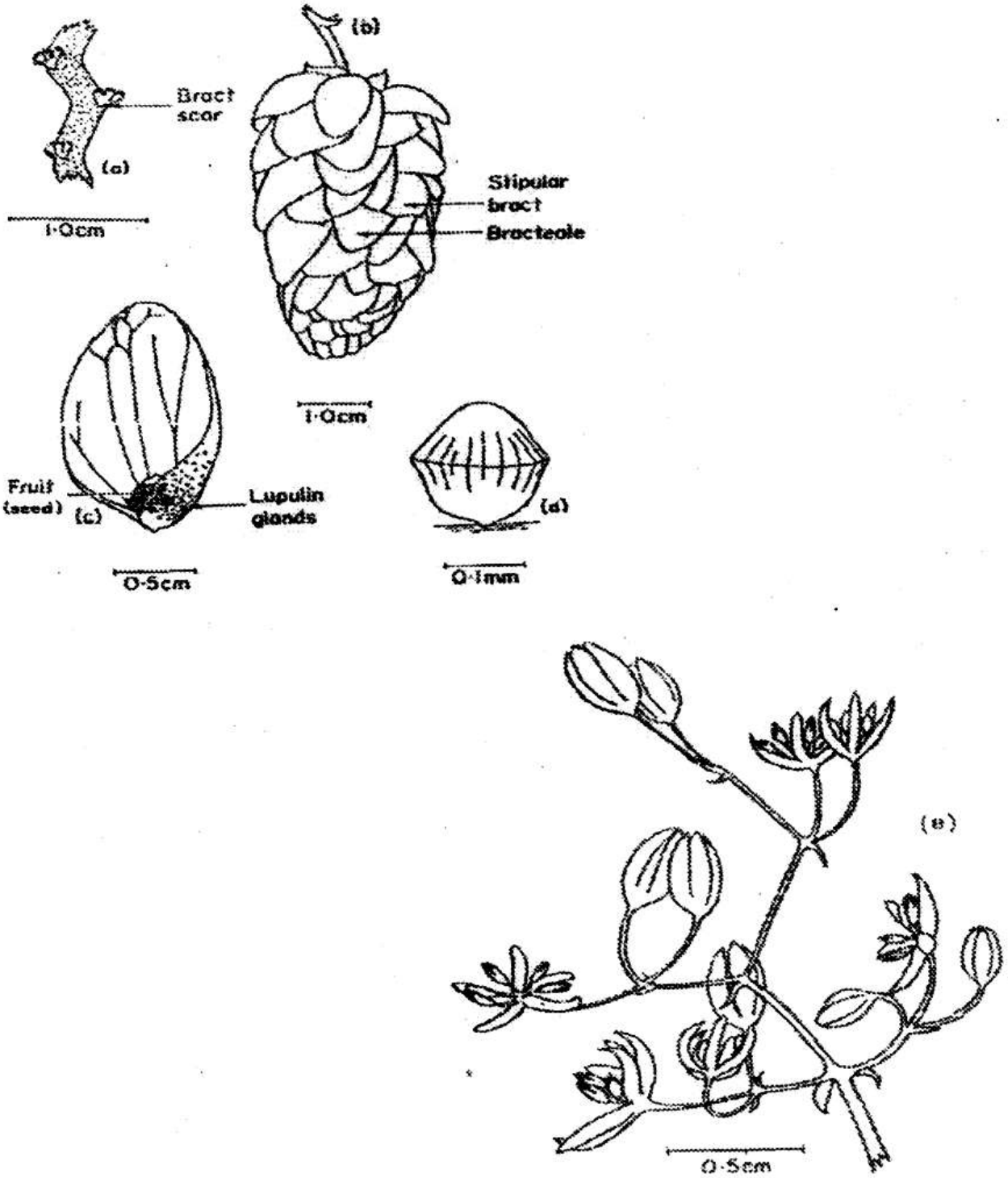


Fig. 2.1: Hop *(Humulus lupulus L)* (a) part of axis ('strig') of cone; (b) single mature hop cone, (c) bracteole with seed and lupulin glands (d) lupulin gland (e) male hop flowers). (Source: Burgess, 1964)

The three major components of alpha acids are humulone, cohumuloe and adhumulone. The beta acids include lupulone, colupulone, and adlupulone which are only marginally bitter (Hough *et al*., 1982).

The structures of alpha acids and beta acids as given by Peacock are shown in Figs. 2.2 and 2.3 respectively.

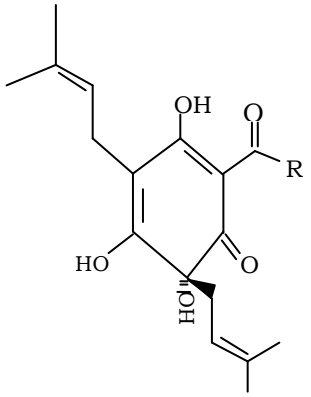


Fig.2.2 Structure of Alpha acids.

Table 2.1: Alpha acids and % alpha acids before isomerization

Alpha-Acid	R=	% of Alpha-acids
Humulone, $C_{21}H_{30}O_5$	Iso-butyl $CH_2CH(CH_3)_2$	40 – 80
Cohumulone, $C_{20}H_{28}O_5$	Iso-propyl $CH(CH_3)_2$	17 – 50
Adhumulone, $C_{21}H_{30}O_5$	Sec-butyl $CH(CH_3)CH_2CH_3$	5 – 15

(Source: Peacock, 2009)

In Table 2.1, if R is CH_2 $CH(CH_3)_2$, then the alpha-acid in Fig. 2.2 is Humulone but if R is $CH(CH_3)_2$, the alpha-acid is Cohumulone while if R is $CH(CH_3)CH_2CH_3$, the alpha-acid is Adhumulone. Similarly, in Table 2.2, if R is $CH_2CH(CH_3)_2$, then the β-acid in Fig. 2.3 becomes Lupulone but R is $CH(CH_3)_2$ represents Colupulone and R is $CH(CH_3)CH_2CH_3$ denotes β-acid as Adlupulone.

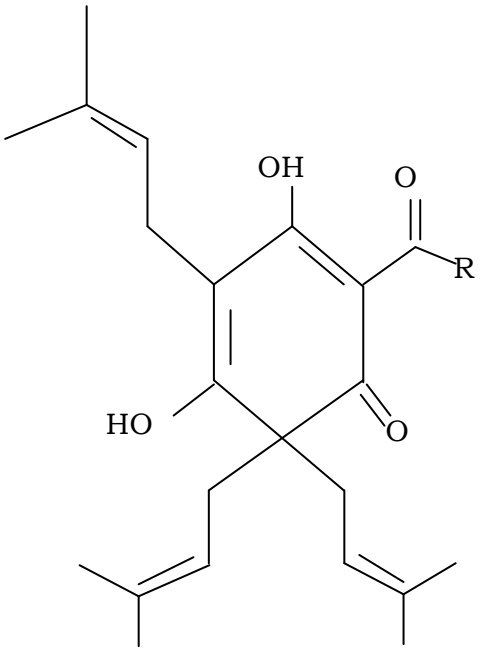


Fig. 2.3: Structure of Beta Acids

Table 2.2: Beta acids and % beta acids before isomerization

Beta-Acid	R=	% of Beta-acids
Lupulone, $C_{26}H_{38}O_4$	Iso-butyl $CH_2CH(CH_3)_2$	15 – 60
Colupulone, $C_{26}H_{36}O_4$	Iso-propyl $CH(CH_3)_2$	35 – 80
Adlupulone, $C_{26}H_{38}O_4$	Sec-butyl $CH(CH_3)CH_2CH_3$	5 – 15

(Source: Peacock, 2009)

When hops are added to the boiling wort in the kettle, their alpha acid go through isomerization and are converted to iso-alpha acids (Fig. 2.4) (Peacock, 2009).

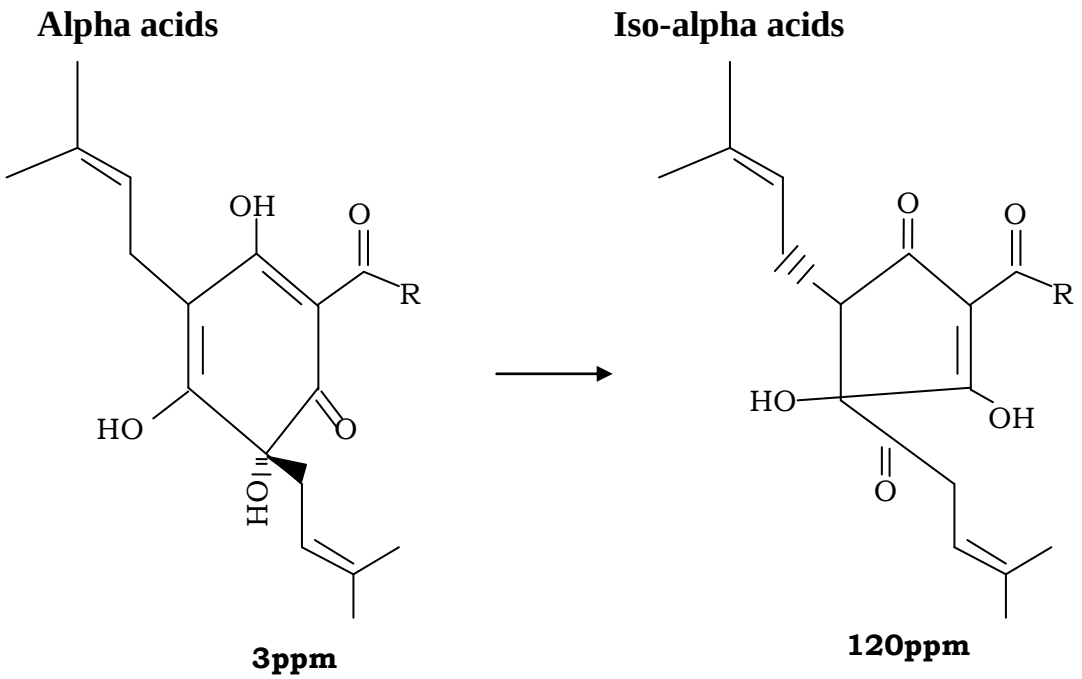


Fig. 2.4: Alpha acids isomerized to Iso alpha acids

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 (Hough *et al*., 1982; Koller, 1969).

In this conversion, humulone is converted to iso-humulone, cohumulone to iso-cohumulone and adhumulone to iso-adhumulonme.

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.

There are many commercial hop varieties, technically known as cultivars, each with its own spectrum of characteristics (Shellie *et al*., 2009). These authors have carried out varietal characterization of hops, namely Willamette, Victoria, Pride of Ringwood, Cascade, Southern Hallertau, Millennium, Southern Saaz and Superpride. Their study was designed as a pilot project for the utilization of metabolites profiling to support the Australian Hop breeding program and the investigators were encouraged by the ability to discriminate different varieties, which exhibited different characteristics from a beer making point of view. The authors averred that it should be anticipated that understanding the significance of various metabolites identified and the differences in metabolite composition between varieties would support decision-making in the breeding program.

Traditional classification of hop cultivars divided them into three groups: (i) high alpha-acid (ii) intermediate alpha-acid and (iii) aroma (noble) varieties (Verzele and de Kenkeleire, 1991). Varieties of hops are chosen for the properties of bitterness, flavour, or bouquet which they will lend to the beer.

Hop products include hop powders, hop extracts and hop oils. Three types of hop powders have been defined by Hop Liaison Committee. Hop powder is any preparation made by grinding hops without any mechanical concentration; enriched hop powder is any preparation made by grinding hop, with some mechanical concentration, and lupulun is a technically pure preparation of lupulun glands. Hop Liaison Committee (1967) also defined hop extracts as any

preparation prepared by solvent extraction of hops, and isomerized hop extracts as any preparation prepared by solvent extraction of hops in which the alpha acids have been isomerized.

Many solvents have been used to extract the brewing principles of hops; amongst others hexane (b.p 69^{o}C), methanol (b.p 64^{o}C, methylene chloride (b.p. 40^{o}C) and trichloroethylene (b.p. 87^{o}C) have been used commercially (Hough *et al*., 1982; Alderton *et al*., 1954). The solution of resins and essential oils obtained is concentrated in a cyclone evaporator, which minimizes heating time, and the final traces of solvent and the more volatile essential oils are removed by heating the residue in a vacuum. The extract is obtained as viscous green syrup. The major question over the use of hop extracts is the problem of solvent residues which, if present, introduces foreign substances into the beer. Not only may they give an unacceptable taint to the beer but many commercial solvents are toxic. The solvent used to produce hop extracts nowadays is preferably liquid CO_2 or ethanol. The two solvents now used are both particularly well suited to the extraction of hops because they dissolve the hop resins and oils completely (Kunze, 1999).

2.1.3 Brewing Water

The mineral content of brewing water has long been recognized as making an important contribution to the flavour of beer (Goldamer, 2008). This is especially important since more than 90% of the beer is water (O'Rourke, 1998). Brewers

interested in brewing a particular beer style first need to evaluate whether or not their water is suitable by comparing it to the water used to produce the beer style in the region of its origin. For example, the water of Dublin for Stouts, the water of Burton-on-Trent for dry, hoppy pale ales and Munich water for darker, mellower lagers.

2.1.4 Brewers' Yeast

Yeast is one of the most important ingredients in beer brewing. It is responsible for metabolic processes that produce ethanol, carbon dioxide, and a whole range of other metabolic by-products that contribute to the flavour and finish of beer (Kramer, 2006). There are literally hundreds of varieties and strains of yeast. In the past, there were two types of beer yeast; ale yeast (the "top fermenting" type, *Saccharomyces cerevisiae)* and the lager yeast (the "bottom-fermenting" type, *Saccharomyces carisbergenis or Saccharomyces uvarum*). Top fermenting yeasts produce beers that are more estery, fruity and sometimes malty, whereas bottom-fermenting yeasts give beers a characteristic sulphurous aroma. Top-fermenting yeasts are used for brewing ales, stouts, porters etc, while bottom-fermenting yeasts are used for brewing lagers such as Gulder, Star, Pilsner, Hero etc.

2.2 BREWING PROCESSES

Malting, malt milling, mashing, wort separation, wort boiling, wort cooling and aeration, beer fermentation, beer conditioning, beer filtration and beer

carbonation are the major processes involved in beer brewing (Kramer, 2006; Palmer, 2006).

The first step in beer brewing is malting. Malting serves the purpose of converting insoluble starch to soluble starch, reduces complex proteins, generates nutrients for yeast and enzyme development (Bamforth, 1985). The three major steps in the malting process include steeping, germination and kilning. The purpose of steeping is to evenly hydrate the endosperm mass and to allow uniform growth during germination (Goldamer, 2008). Steeping begins by mixing the barely kernels with water to raise the moisture level and activate the metabolic processes of the dormant kernel. After some days, the grain begins to germinate. During germination, enzymes within the grain convert the hard, starchy interior of the grain to maltose (Kneen, 1994). At this point, the grain is called malt. After several days, when the bulk of the starch has been converted to sugar, the malt is heated and dried. This process called kilning stops the malt from germinating any further. A portion of the malt may be further roasted to varying degrees of colour and flavour to create different styles of beer (Goldamer, 2008).

The kilned malt is processed in a mill which cracks the husk (the outer coating of the grain). The objective of milling is to reduce the malt to particle sizes which will yield the most economic extract (wort) and will operate satisfactorily under brew house conditions and throughout the brewing process. The more extensive the malt is milled, the greater the extract production (Ault and Newton, 1974).

After milling, the milled malt is transferred to a temporary storage hopper commonly called the grist case, which feeds the mash tun.

Mashing involves mixing milled malt and solid adjuncts (if used) with water at a set temperature and volume to continue the biochemical changes initiated during the malting process. The malt and adjunct particles swell, starches gelatinize, soluble materials dissolve, and enzymes actively convert the starches to fermentable sugars (Gutcho, 1976). The end result according to Gutcho is wort with a fixed gravity, sugars and proteins (soluble and non soluble) that affect physical and biochemical changes during fermentation.

The composition of the wort varies according to the style of the beer. The mashing process is conducted over a period of time at various temperatures in order to activate the enzymes responsible for the acidification of the mash and the reduction in protein and carbohydrates. The principal enzymes responsible for starch conversion are alpha and beta-amylases. The conversion of starch molecules by these enzymes into fermentable sugars and unfermentable dextrins is the most important change that takes place during mashing (Hough *et al*., 1982). Biochemical processes by mashing and characterization of the fermentation of feed barley during brewing had been studied by Georgieva and others (Georgieva *et al*., 2007).

After mashing, when the starch has been broken down, the next step is to separate the liquid extract, the wort from the residual undissolved solid materials found in the mash. Wort separation is important because the solid contain large

amounts of proteins, poorly modified starch, fatty materials, silicates and polyphenols (tannins) (Hough *et al.*, 1982). The objectives of wort separation (lautering) are to produce clear wort and obtain good extract recovery. The method of separating the wort from the mash solids and the equipment used is mainly a matter of choice on the part of individual brewer. Wort separation is carried out by a number of different methods: the mash tun, the lauter tun, the mash filter, or the strainmaster (Hudson, 1997). The lauter tun is internationally accepted and continues to be the predominant wort separation device (Andrews, 2004).

Sparging extracts the fermentable liquid, known as wort, from the mash. Sparging begins just after the first wort has been collected and after the mash has settled but before the surface of the mash has become too dry. The sparge water (75-78^{o}C) (Briggs *et al.*, 2004) should be applied at a rate that matches the runoff rate and is applied via the sparge arms that are centrally located. As the first wort is drawn off, sparging is initiated before the wort level reaches the top of the grain bed.

Following wort separation and extraction of the carbohydrates, proteins, and yeast nutrients from the mash, the clear wort must be conditioned by boiling in the kettle. In this stage called brewing, the wort is transferred to a large brew kettle and boiled for up to two hours. Boiling effectively sterilizes the wort to kill any bacteria that may spoil the wort during fermentation (Busch, 1997; Hudson,

1970). At this stage, hops are added to the wort to provide a spicy flavour and bitterness that balances the sweetness of the wort. The type of hops used and the length of time they are boiled are determined by the style of the beer (Sidor, 2006). To produce a beer with a stronger bitter flavour, hops are boiled for at least 30 minutes, and often longer. This enables the bitter acids and essential oils in the hop to infuse into the wort. The purpose of wort boiling is to stabilize the wort and extract the desirable components from the hops. The principal changes that occur during wort boiling include sterilization, enzyme inactivation, protein precipitation and colour development, isomerization, dissipation of volatile constitutions, concentration of wort by evaporation and reduction in wort pH to a final pH of between 5.2 and 5.3 (Comrie, 1967; Thomas and Neve, 1976). After the boil, the next step is to separate the hop debris and the trubaceous matter (hot break) so that the wort is bright and clear before cooling.

As the clear wort is cooled, the previously invisible coagulum loses its solubility and precipitates. The precipitate is referred to as cold break and begins forming at about 60^{o}C. The cold break mostly consists of protein-polyphenol (tannins) complexes whereas the hot break is mostly proteinecious. The cold break also has a higher level of carbohydrates than the hot break (Busch, 1997). After the wort is cooled, the break must be removed before fermentation else the beer will taste wort-like, bitter and even harsh. Fix and Laurie (1997) observed that cold break removal aids in colloidal stability in the beer, circumvents the formation of sulphury flavours, and removes harsh bitter fractions derived from hops. De

Clerck and de Dijeker (1957) had earlier suggested that clearer worts lead to beer that clarifies better. The clear cooled wort is aerated to increase yeast activity and to start fermentation process.

The yeast strain itself however is a major contributor to the flavour and character of the beer (Van Der Aar, 1995). Thus, the choice of yeast strain depends on such things as the oxygen requirements, cropping methods, attenuation limits, fermentation rates, fermentation temperatures, flocculation characteristics and the flavour profile (Freeman, 1999). Yeast can be directly added to the fermenter (batch) or mixed with the wort in a starter tank prior to transfer to the fermenter.

Fermentation is the process by which fermentable carbohydrates are converted by yeast into alcohol, carbon dioxide, and numerous other byproducts. It is these byproducts that have a considerable effect on the taste, aroma and other properties that characterize the style of beer (Tenney, 1985).

There are many different fermentation systems that are used worldwide that have evolved based on available technology, brewing materials and perceived product quality. Rehberger and Gary (1995) noted that some of the more common fermentation systems in use today include cylindroconical, traditional ale top-skimming system, traditional ale dropping system, Yorkshire square system, Burton Union system, open square fermenters and dual purpose vessel system.

Following primary fermentation, the “green” or immature beer is far from finished because it contains suspended particles, lacks sufficient carbonation,

taste and aroma, and it is physically and microbiologically unstable. Conditioning reduces the level of these undesirable compounds to produce a more finished product. The component processes of conditioning are maturation, clarification and stabilization. Traditionally, maturation involves secondary fermentation of the remaining fermentable extract at a reduced rate controlled by low temperatures and a low yeast count in the green beer. During secondary fermentation, the remaining yeast becomes re-suspended, utilizing the fermentable carbohydrate in the beer. The carbohydrate can come from the residual gravity in the green beer or by addition of primary sugar or by kraeseing (the infusion of a strongly fermenting young beer into a larger volume of beer that has undergone primary fermentation to start a secondary fermentation). Yeast activity achieves carbonation, purges undesirable volatiles, removes all residual oxygen, and chemically reduces many compounds thus, leading to improved flavour and aroma (Grant, 1979).

Today, the use of modern equipment for refrigeration, carbonation and filtration obviates the need for secondary fermentation and a long cold storage. The green beer undergoing cold storage is fully attenuated and virtually free from yeast which is achieved because of higher fermentation temperature and diacetyl rest.

Although, much of the suspended yeast will settle to the bottom of the storage tank by sedimentation, it can be time consuming in preparing beer for filtration. Consequently, MacDonald (1984) investigated the addition of fining agent such

as gelatin during storage. He observed that the addition of fining agents speed up sedimentation. Alternatively, they used centrifugation to remove yeast and other solids after fermentation and concluded that any of these processes clarifies the beer.

In addition to clarification (i.e. removal of yeast), beer must display physical stability with respect to haze. Colloidal instability in beer is caused mainly by interactions between polypeptides and polyphenols. Reducing the levels of one or both of the precursors using suitable stabilizing treatments extends the physical stability of the beer (Thomas, 1997).

Although, conditioning plays important role in reducing yeast and haze loading materials, a final beer filtration is needed in order to achieve colloidal and microbiological stability. The beer must be rendered stable so that visible changes do not occur during shelf life.

The next major process that takes place after filtration and prior to packages is carbonation. Carbon dioxide not only contributes to perceived fullness or ‘body’ and enhances foaming potential, it also acts as flavour enhancer and plays an important role in extending the shelf life (Briggs, 1989).

Once the final quality of the beer has been achieved, it is ready for packaging. Beer packaging can be done by either kegging or bottling. Kegging involves filling carbonated pasteurized beer into sterile aluminum or stainless steel kegs of various sizes. The layout of the bottling line will depend on a number of factors

but typically consists of a series of processes such as beer sterilization, bottle feeding, bottle rinsing, bottle filling and post bottle filler operations. Post bottle filler operations include crowning, post rinse, bottle drying, bottle labeling and case packing (Goldamer, 2008).

2.3 NIGERIAN PLANTS

2.3.1 *Garcinia kola* (Bitter kola)

Garcinia kola, an angiospermae, belonging to the family *Guttiferae*, is known in commerce as bitter cola. On chewing, *G. kola* has a bitter astringent and resinous taste, somewhat resembling that of raw coffee, followed by a slight sweetness. 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 (Eisner, 1990).

Garcinia kola is a dicotyledonous plant found in moist rain forests and swamps and grows as a medium sized tree up to a height of about 12m high. It is cultivated through the seedlings or with cuttings. It is found in countries across West and Central Africa and distributed by man around the towns and villages in such countries like; Nigeria, Ghana, Cameroun, Angola, Sierra Lone, Togo, Congo Democratic Republic, Liberia, Gambia etc. Across the places where it grows, it is known by different names such as bitter kola, male kola (English),

Orogbo (Yoruba), *Aku ilu* (Igbo) and *Mijin goro* (Hausa). It is known as false kola mainly due to the absence of stimulants which characterize the kola nut seeds. It is also known as male kola due to the reported aphrodisiac properties of *Garcinia kola* (Braide, 1991).

Garcinia kola is regarded as a wonder plant because every part of the plant (bark, leaf, root, wood, seed) has been found to be of medicinal importance. The medicinal importance of bitter cola is based mainly on the phytochemical components of the palnt. From its roots to its leaves, the plant is known to contain several phytochemicals noted for their medicinal importance (Iwu *et al*., 1990).

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 hepatoxins (Terashima *et al*., 2002). *Garcinia kola* seeds are also used in the treatment of diabetes, bronchitis and throat infections as well as treatment of liver disease and diarrhea (Iwu *et al*., 1990; Braide, 1991). Traditionally, the plant is used as a natural antimicrobial. Other medicinal properties of the plant include its usage in the treatment of skin infection in Liberia and Congo Democratic Republic. The powdered bark of the plant is applied to malignant tumors, cancers etc. The plant latex is taken internally for

gonorrhea and externally to seal new wounds and prevent sepsis (Adesuyi *et al*., 2012).

In Congo, a bark decoction is taken for female sterility and to ease child birth, the intake being daily till conception is certain and then at half quantity throughout the term. The bark is added to that of *Sarcocephalus latifolinus* which has a strong reputation as a strong anti-diuretic, in the treatment of urinary decongestion and chronic urethral discharge. In Ivory Coast, a decoction of the bark is taken to induce the expulsion of a dead foetus, while the seed and the bark are taken for stomach pain (Jackson, 2000). In Sierra Leone, the roots and the bark are taken as a tonic for sexual dysfunction in men (Guisseppe and Baratta, 2000). The bark is also added into palm wine to improve its potency.

In Nigeria, a cold water extract of the roots and bark with salt are administered to cases of bronchial asthma or cough, or vomiting (Iwu *et al*., 1990). The medicinal properties of bitter cola can be classified under purgative, antiparasitic and antimicrobial. The use of *G. kola* as hop substitute in tropical beer brewing has been investigated (Ajebesone and Aina, 2004).

2.3.2 *Azadirachta indica* (Neem)

Azadirachta 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 (Keay *et al*., 1964).

The name *Azadirachta* is the Persian vernacular name of this genus. It is native to India, Myanmar, Bangladesh, Sri Lanka, Malaysia and Pakistan. *Azadirachta* is the well known 'Neem' tree in Eastern India, where it occurs wild and also much planted. It grows in tropical regions and is known by many different names including *Azad Dirakht* (Persian), *Neeb* (Arabic), *Dogon Yaro* (in some Nigerian languages), Indian Lilac (English), *Paraiso* (Spanish) and *Maurabain* (Swahili) (Pennington and Style, 1975).

The tree grows up to 24m high but usually smaller in Nigeria, with dense, wide spreading crown; the bole is short and stout while the bark is dark, rough with wide shallow longitudinal fissure separated by more or less flat ridges; the leaves have 5-8 pairs of leaflets, up to 10cm long by 3cm broad, lanceloate, falcate, very asymmetrical and glaborous; and the margin is coarsely toothed, rarely lobed and the apex is long acuminate (Keay *et al*., 1964). The genus is evergreen, but in serious drought, it may lose most or nearly all of its leaves.

Products made from Neem have been used in India for over two millennia for their medicinal properties. They are found to be antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative (Buttler and Bailey, 1973; Mabberley, 1995). Joshi and other researchers examined phytochemical extraction and antimicrobial properties of different medicinal plants including *Azadirachta indica* (Joshi *et al*., 2010).

2.3.3 *Vernonia amygdalina* (Bitter leaf)

Vernonia amygdalina is a shrub or small tree with petiolate leaf of about 6mm in diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste (Igile *et al*., 1995). No seeds are produced and the tree is distributed through cutting (Aregheore *et al*., 1997).

Vernonia amygdalina grows under a range of ecological zones in Africa and produces large mass of forage and is drought tolerant (Akinkpelu, 1999). There are about 200 species of *Vernonia*.

The leaves are washed before eating to get rid of the bitter taste. They are used as vegetable and to stimulate the digestive system, as well as reduce fever (Onwuka *et al*., 1989). Furthermore, they are used as local medium against leech which transmits bilharziasis (Fayemi, 1982).

The use of bitter leaf (*Vernonia amygdalina)* as local substitute for hops in the Nigerian Brewing Industry had been investigated by Adama and others (Adama *et al*., 2011).

Okpoko, (2010) studied the use of V*ernonia amygdalina* extract as means of extending the shelf life of locally brewed sorghum beer in Nigeria. *Vernonia amygdlina* has been observed to be eaten by goats in central zone of Delta State, Nigeria. However, in general, it has been found that V*ernonia amygdalina* has an astringent taste which affects its intake (Fayemi, 1982). The bitter taste is due to anti-nutritional factors such as alkaloids, saponnins, tannins and glycosides (Buttler and Bailey, 1973; Olsztyn *et al*., 1994). V*ernonia amygdalina* has also

been fed to broilers where it was able to replace 300g Kg^{-1} of maize-based diet without affecting feed intake, body weight gain and feed efficiency (Tegula *et al*., 1993).

2.3.4 *Gongronema latifolium* (Heckel)

Gonogronema latifolium is a climbing shrub of the family *Asclepiadeceae*. It is wide spread in tropical Africa and thrives in Senegal, Cameroun, Nigeria, Chad, Sierra Leone and Democratic Republic of Congo.

Gongronema latifolium grows up to 5m long, stems hollow, all parts soft-hairy to glaborous, 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.

This species occurs in rainforest, deciduous and secondary forests, from sea level up to 900m altitude. It can be propagated by seed or soft wood, semi-hardwood and hardwood cuttings.

Few chemical analyses have been performed on *Gongronema latifolium*. From the leaves, several 17β-Marsdenin derivatives (pregnane glycosides) were isolated (Akuodor *et al*., 2010) as well as β-sitosterol, (Burkill, 1985), lupenyl cinnamate, lupenyl acetate and lupeol (Eleyinmi *et al*., 2008).

Different methanolic and ethanolic leaf extracts showed promising hypoglycaemic and antihyperglycaemic activities in a dose on normal and alloxan-induced or streptozotocin-induced diabetic rabbits (Irvine, 1961).

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 (Dike, 2010; Emeka and Obiora, 2009; Nwanjo and Alumanah, 2006; Okolie *et al*., 2008; Oliver-Bever, 1986).

The leaves of *Gongronema latifolium* were tested as possible hop substitute for brewing beer (Okafor and Anichie, 1982). Water extracts of the powdered leaves gave low bittering values, but extraction of the powdered leaves with organic solvents significantly increased analytical bitterness of levels comparable with hops. The leaf extracts also had antimicrobial properties comparable with acetone extracts of hops (Okafor, 2007).

Gongronema latifolium is widely used in West Africa for medicinal and nutritional purposes. An infusion of the aerial parts is taken to treat cough, intestinal worms, dysentery, dyspepsia and malaria (Uko, 1988). It is also taken as a tonic to enhance appetite. In Sierra Leone, an infusion or decoction of the stems with lime juice is taken as a purge to treat colic and stomach ache (Usher, 1974). In Senegal and Ghana, the leaves are rubbed on the joints of small children to help them walk (Sharp and Laws, 1981). The boiled fruits in soap are eaten as a laxative. In Nigeria, a leafy stem infusion is taken to treat diabetes and blood pressure (Uko, 1988). The latex is applied to teeth affected by caries. It is also taken for controlling weight gain in lactating women and overall health

management (Uko, 1988). Asthma patients chew fresh leaves to relieve wheezing. A cold maceration of the roots is also taken as a remedy for asthma. A maceration of the leaves in alcohol is taken to treat biharzias, viral hepatitis and as a general antimicrobial agent (Onyeike and Osuji, 2003).

In Southern Nigeria, the Igbo call the leaf *utazi* and the Yoruba *arokeke*. They are widely used as a leafy vegetable and as a spice for sauces, soup, and salads. The leaves are used to spice locally brewed beer. In Sierra Leone, the pliable stems are used as chew sticks. Generally, *Gongronema latifilium* is an important medicinal plant, vegetable and spice.

2.4 PROPERTIES OF BEER

2.4.1 Physical and chemical properties of beer

Beer is a complex mixture; over 400 different compounds (Hough *et al*., 1982) 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 beer and lagers 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 (Hough *et al*., 1982).

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. 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 (Grant, 1977).

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 (Grant, 1977).

Methods for beer analysis have been published by, inter alia, the European Brewery Convention-*Analytica* (EBC, 1975), the American Society of Brewing Chemists (ASBC, 1976) and the Institute of Brewing (IB, 1977). Not all of the methods described refer to the estimation of specific constituents. Some give

method, 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 (CO_2), volume of sulphur (VI) oxide (SO_3), dissolved oxygen, colour, haze, foam head retention and viscosity (Hough *et al*., 1982, Fix, 1989). 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 (Meilgaurrd and Peppard, 1986).

Carbon dioxide is a natural product of fermentation and beers should contain 3.5 – 4.5g/*l*. De Clerck (1957) stated that 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.

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 (MacWilliam and Philips, 1959). 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 (Bamforth, 1985). A good head of dense stable foam on a glass of beer is visually appealing and such beer always has ‘mellow’ palate (De Clerck, 1957). 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 CO_2 (Zurcher and Kursawe, 1973; Parker and Richardson, 1970; Carroll, 1979).

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. 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 (Wainwright, 1971).

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 (Kraus-Weyerman, 1998).

2.4.2 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.

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. 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 (Hough *et al*., 1982).

Flavour has been described as complex sensation comprising taste, odour, roughness or smoothness, hotness or coolness, pungency or blandness (Moncrieff, 1967). 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 melanoidins in the beer.

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^{0}C) than those produced by top fermentation (10 – 20^{0}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, melanoidins and the principal amino acids in beer, proline (Hough *et al*., 1982).

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 possess 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 (Hough *et al*., 1982). They occur on the back, tip and sides of the tongue.

The brewer and his customer make a subjective assessment of beer flavour each time they taste but for a more objective appraisal, it is usually desirable to submit the beer, with suitable controls, to a taste panel. Taste panel may be used to:

i. Select qualified judges
ii. Correlate sensory with chemical and physical measurements
iii. Study processing effects, maintain quality, evaluate raw material selection, establish storage stability, and reduce costs.
iv. Evaluate quality and
v. Determine consumer reaction (Amerine *et al*., 1965).

The types of test used include:

i. Difference tests
ii. Rank order
iii. Scoring tests
iv. Descriptive tests
v. Hedonic scaling
vi. Acceptance and preference tests (Amerine *et al*., 1965).

Difference tests are most commonly used in brewing industry, the results of which are readily analyzed by statistics. Several forms of difference tests are used. The **'A**-not-**A'** form of test is perhaps the simplest. Assessors are first familiarized with a standard **A** and then presented in a random manner, either with **A** again or with the comparative sample **B**. In the paired-comparison test, two samples are presented simultaneously **(AA, AB, BA,** or **BB)** and assessors report either 'there is a difference', or there is no difference. The duo-trio test is a three-glass test in which the reference sample is specified and the assessor is required to say which of the others is the same as the reference .In the triangular test, the same beer is presented in two glasses and a different beer in the third. The assessor is asked to identify the odd sample. However, tasters may find difficulty in finding the odd beer from certain of the six possible arrangements **(AAB, ABA, ABB, BAA, BAB** and **BBA)** when one of the samples has a lingering flavour or after taste (Clapperton, 1975).

The beers presented to the panel should be at the same temperature, in the same condition (CO_2 content) and served in opaque glasses or in a darkened room to eliminate differences in colour or clarity. Each taster is provided with a form (Table 2.3) (EBC, 1975) on which he must record his result immediately after tasting. No discussion of results is allowed until all the tasters have completed their report forms. Each taster's findings are recorded on summary sheet and the significance of the results evaluated by reference to Bengtsson's Table (Appendix 1).

Table 2.3: Taste Panel report forms. *Analytica*.

INDIVIDUALTWO-GLASS FLAVOUR TESTING FORM

Name of Taster _____ Date: _____

Beers compared _____

Is there a flavour difference between the two? _____

Can you describe the difference? *optional*_____

Which beer do you prefer (if either)? _____

INDIVIDUAL THREE-GLASS FLAVOUR TESTING FORM

Name of Taster _____ Date: _____

Beers compared _____

Which is the number of the different glass of beer? _____

Can you describe the difference? *optional*_____

Which beer do you prefer (if either)? _____

Source: EBC, 1975.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

Hop leaves were purchased from Youngs Ubrew Goldings Hops, Bilston, West Midland WV, United Kingdom (148DL – http://www.youngubrew.co.uk). Isomerised hop extract was also purchased from Ritchies, Rolleston Road, Barton-on-Trent, Staffs England, DE130JX, United Kingdom (http://www.Ricthieproudcts.co.uk). The leaves of *A. indica, G. latifolium, V. amygdalina* and the seeds of *G. kola* were obtained from Pope John Paul II Major Seminary Botanical Garden at Okpuno, Awka South Local Government Area of Anambra State. The plants were further identified by taxonomists in the herbaria of two different institutions namely Department of Botany, Nnamdi Azikiwe University, Awka and Department of Forestry, Anambra State Ministry of Environment, Awka.

Chemicals used in the analyses, all of analytical grade quality were supplied as follows:

a. Sigma – Aldrich, Germany
    (i) Hydrochloric acid
    (ii) Tetraoxosulphate (VI) acid
    (iii) Trioxonitrate (V) acid
    (iv) Orthophosphoric acid
    (v) Perchloric acid

b. Riel – deHaen, Germany
    (i) Methanol

(ii) Ethanol

(iii) Petroleum ether (40-60^{o}C)

(iv) Isobutyl alcohol

c. Fisher Scientific, London

(i) Sodium chloride

(ii) Starch soluble

(iii) Phenolphthalein

(iv) Methyl red

(v) Casein solution

(vi) Bromothymol blue

Other chemicals of non analytical grade were obtained from:

a. Duchefe, Haarlem, the Netherlands

(i) Dipotassium hydrogen phosphate

(ii) Potassium dihydrogen phosphate

(iii) 3, 5 – dinitrosalicylic acid

(iv) Trichloroacetic acid

b. BDH Chemicals Poole, England

(i) Ammonium hydroxide

(ii) Ethanoic acid– Glacial

(iii) Ammonium thiocyanate

(iv) Potassium iodide – Analar

(v) Silver trioxonitrate (V)-Analar

(vi) Iodine solution

(vii) Iron (III) chloride

c. May and Baker (M&B), England

(i) Calcium chloride dihydrate

(ii) Calcium oxalate dihydrate

(iii) Potassium tetraoxomanganate (VII)

(iv) Sodium hydroxide

(v) Sodium bicarbonate

The non analytical grade chemicals were purified by standard procedure when required.

Other materials such as special filter papers (Nos. 1, 11 and 42) were supplied by Whatman.

Also included in the list of materials are thermostated water bath (G. Bosh, DK420), vacuum oven (Techmel & Techmel, TT-9053), muffle furnace (Searchtech, SX 2-5-12), laboratory milling machine (Gibbons, Model 8), top loading balance (Mettler Toledo, HH-S), orbital centrifuge (Anke, TDL-5-A), pH meter (Searchtech, PHS-3C), rotary evaporator (PEC medical,RE52-2), Atomic Absorption Spectrophotometer, A.A.S (Buck Scientific,V210-VG), Gas Chromatography/Mass Spectrometer-GC/MS (Schimadzu, Q2010 PLUS), U.V-visible spectrophotometer (PEC medical,UV752) and Moisture Analyser (Satorius, SR-75DHG).

3.2 METHODS

3.2.1 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 and ethanolic extracts by steeping procedure.

3.2.2 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.

3.2.3 Ethanol Extraction

The ethanol extract was prepared by steeping 300g of the dry powdered plant material in 1.5 litres of ethanol 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 60^{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 use.

3.2.4 Thin Layer Chromatography (TLC)

The plate was prepared by smearing 20% aqueous slurry of silica gel in water on a precleaned and dried chromatographic plate (3.5cm x 7.5cm). This was dried in an oven at 105^{o}C for 1 hour. At the distance of 1.5cm from the bottom of the thin layer plate was drawn a horizontal line with pencil (2H) at which position the extract was spotted. This was inserted into the chromatographic tank and 25ml of the mobile solvent (CH_3OH) was introduced carefully down the side of the tank. The sample was eluted for 45 minutes after which the plate was put into a big bell jar wherein the colour of the fractions were developed using iodine crystals.

3.2.5 GC-MS Analysis

3.2.5.1 GC-MS Technique

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.

3.2.5.2 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. This process is demonstrated by the GC chromatogram of isomerized hop extract shown in Appendix 3F. The conditions used to obtain the chromatogram are presented as foot notes below the chromatogram.

Furthermore, there are 14 chromatographic peaks in the GC chromatogram of processed female hop inflorescence and each peak represents a chemical

constituent. Line#: 1 is the mass spectrum of peak 1 in the chromatogram. The analysis of this peak by the MS is presented in Hit#: (1-5). In this presentation, the closest simulated peak pattern is Hit#: 2. The instrument gives the systematic name of the constituent as 4, 4-dimethyl-2-buten-4-olide. Similarly, the other chromatographic peaks are analysed and named accordingly. This procedure was applied to the five methanolic extracts. The GC-MS results are annexed as Appendix 2(A-F).

3.2.6 Phytochemical Analysis

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 analyzed include: alkaloids, tannins, saponins, oxalates, phytatic acid, trypsin inhibitors, cardiac glycosides, haemagglutinins, cyanogenic glycosides and hydrogen cyanide. All these were determined based on methods of analysis described by AOAC (1980) as adopted and described by other researchers Sofowora (1993); Trease and Evans (1989) and Harbone (1995). Use was made of the methanolic extracts in the phytochemical screening.

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).

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

Tannins

To determine tannin, the Butler (1989) titration method as described by Viji and Parvatham (2011) 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,

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

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).

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

Oxalates

This was determined according to Harbone (1995) as adopted by Osagie *et al*. (1996). This determination involves three major steps – digestion, oxalate precipitation and permanganate titration.

Digestion: 2g of the extract was suspended in 190cm^3 of distilled water in a 250cm^3 volumetric flask. 10cm^3 of 6M HCl was added and the suspension digested at 100^oC for 1 hour. The digested solution was allowed to cool, filtered and the filtrate received in another 250cm^3 volumetric flask. The residue was washed twice with 10cm^3 of deionized water and made up to the mark.

Oxalate Precipitation: Aliquot portions (125cm^3) of the filtrate were measured into two different beakers and four drops of methyl red indicator added to each beaker. This was followed by the addition of ammonia solution (dropwise) until the test solution changes from salmon pink colour to a faint yellow colour (pH 4 – 4.5). Each portion was then heated to 90^oC, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was heated to 90^oC again and 10cm^3 of 5% $CaCl_2$ solution was added with constant stirring. After heating, it was cooled and left overnight at room temperature. The solution was then centrifuged at 2500 rpm for 5 minutes. The supernatant was decanted and the precipitate completely dissolved in 10cm^3 of 20% (v/v) H_2SO_4 solution.

Permanganate Titration: The total filtrate resulting from digestion of 2g of the sample extract was made up to 300cm^3. Aliquots of 125cm^3 of the filtrate was heated until near boiling and then titrated with 0.05M $KMnO_4$ solution to a faint pink colour which persists for some 30 seconds. The Calcium oxalate content is calculated using the formula:

$$\frac{\mathrm{T} \times (\mathrm{VMe})(\mathrm{DF}) \times 10^5}{(\mathrm{ME}) \times (\mathrm{Mf})} \quad (\mathrm{ppm})$$

where T is the titre value of $KMnO_4$ (cm^3),

VMe is the Volume – Mass equivalent (i.e. 1cm^3 of 0.05M $KMnO_4$) solution is equivalent to 0.00225 anhydrous oxalic acid).

DF is the dilution factor, $Vt/A = 2.4$ where Vt is the total volume of titrant (300cm^3) and A is the aliquot used (125cm^3)

ME is the molar equivalent of $KMnO_4$ in oxalate and

Mf is the mass of sample used.

Phytatic acid

Phytatic acid contents were determined using the method of Trease and Evans (1989) as adopted by Sofowora (1993).

2g of the different methanolic extracts was each weighed into different 250cm^3 conical flasks. Each sample extract was soaked in 100cm^3 of 2% concentrated HCl for 3 hours. The solutions were then filtered, through a double layer of hardened filter paper. Fifty centimeter cube (50cm^3) of each filtrate was placed in 250cm^3 beaker and 100cm^3 distilled water added to each of them. Ten cubic centimeter (10cm^3) of 0.3% ammonium thiocyanate solution was added as

indicator and titrated with standard iron (III) chloride solution which contained 0.00195g iron per cm^3. The end point was slightly brownish – yellow which persisted for 5 minutes. The percentage Phytic acid was calculated using the formula;

$$\text{Phytic acid (\%)} = \frac{\text{Average titre value } \times 0.00195}{2} \times 100$$

Trypsin Inhibitor

Trypsin inhibitor activator of the samples was determined by the method of Harbone (1995). 2 g of each sample was weighed into a screw-cap centrifuge tube and $10cm^3$ of 0.1M phosphate buffer was added. The contents were shaken at room temperature for 1 hour on a UDY shaker. The suspension obtained was centrifuged at 5000 rpm for 5 minutes and filtered through Whatman No. 42 filter paper. The volume of each was adjusted to 2ml with phosphate buffer. The test tubes were placed in water bath, maintained at 37^oC and $6cm^3$ of 5% tricarboxylic acid (TCA) solution was added to one of the tubes to serve as a blank. Two ml casein solution was added to all the tubes previously kept at 37^oC and were incubated for 20 minutes. The reaction was stopped after 20 minutes by adding $6cm^3$ of TCA solution to the experimental tubes and then shaken. The content was allowed to stand for 1 hour at room temperature. The mixture was filtered through Whatman No. 42 filter paper and the absorbance of filtrate from sample and trypsin standard solutions were read at 410nm.

Percentage trypsin inhibitor is expressed as follow: (Ologhobo and Fetuga, 1983).

$$\% \text{ Trypsin inhibitors} = \frac{A^{r}_{410} - A^{s}_{410}}{A^{r}_{410}} \times 100$$

where

A^{r}_{410} = Absorbance of reference at 410 nanometers

A^{s}_{410} = Absorbance of sample at 410 nanometers

Haemagglutinin

Haemagglutinin level of the samples were determined by the method of Jaffe (1979). Two grams of each sample was weighed and 50cm^3 of solvent of mixture of isobutylalcohol and trichloroacetic acid were added and allowed to shake on a UDY shaker for 6 hours to extract the haemagglutinin. The mixture was filtered through a double layer filter paper and maintained in a water bath for 2 hours at 80^{o}C and the filtrate was allowed to cool. A set of standard solutions of haemagglutinin, ranging from 0 to 10ppm were prepared from haemagglutinin stock solution. The absorbances of the standard solutions as well as that of the filtrate were read at 220nm on a digital spectrophotometer. Haemagglutinin concentration was calculated thus:

$$\text{Haemaglutinin conc.} = \frac{\text{Absorbance of Sample} \times \text{Conc. of Standard}}{\text{Absorbance of standard}}$$

Cardiac Glycoside

The method of Ayoola *et al*., (2006) was employed. To 2g each of the sample extract were added 50cm^3 of distilled water and left for 48 hours, and then

filtered with No. 42 Whatman filter paper. The filtrate was concentrated to a volume of 5cm^3. To 1cm^3 of the extract was added 1cm^3 of 2% solution of 3,5-Dinitrosalicylic acid in methanol and 1cm^3 of 5% aqueous NaOH. The mixture was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered with a pre-weighed filter paper. The filter paper with the residue was dried in an oven to constant weight at 50^{o}C. The weight of the filter paper with residue was taken. The cardiac glycoside was calculated in percentage as:

$$\%\ Cardiac\ glycoside = \frac{(Weight\ of\ filter\ paper + residue) - (Weight\ of\ filter\ paper)}{Weight\ of\ sample} \times 100$$

Cyanogenic Glycoside

10g of the sample extract was weighed into a 500cm^3 round bottomed flask. To the sample extract was added 400cm^3 of distilled water and heated on a bath for 2 hours. Distillation procedure was carried out and about 150cm^3 of distillate was collected in a 250cm^3 conical flask containing 20cm^3 of 2.5% NaOH. To 100cm^3 of the distillate containing cyanogenic glycoside 10cm^3 of 6M NH_4OH and 2cm^3 of 5% KI were added. The mixture was titrated with 0.02M Silver nitrate to a permanent turbidity to indicate the end point.

Cyanogenic glycoside is obtained from the relation:

1cm^3 of 0.02M $AgNO_3$ = 0.54 Cyanogenic glycoside (ppm) (Bradbury *et al.*, 1999).

Hydrogen Cyanide

The hydrocyanic acids (HCN) of the samples were determined using the procedure of Bradbury *et al* (1999). This was done by soaking 2g of each sample in 100cm^3 of water followed by the addition of 10cm^3 of concentrated orthophosphoric acid and left for 16 hours at a temperature of 38^oC. Each sample extract was transferred into a two-necked 500cm^3 flask connected to a steam generator. This was steam-distilled with saturated sodium bicarbonate solution contained in a 50cm^3 conical flask for 60 minutes. To 20cm^3 of each distillate was added 1cm^3 of starch solution. The mixture was titrated with 0.2M iodine solution.

Hydrogen Cyanide concentration was obtained from the relation:

1cm^3 of 0.2M iodine solution = 1.08 HCN (ppm)

3.2.7 Metal 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 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. Twenty cubic centimeter ($20cm^3$) 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.

3.2.8 Brewing of Beer

The processes involved in beer brewing namely malting, mashing and fermentation were employed. Fig. 3.1(a-f) shows the processes involved in beer brewing.

Malting: 400g sorghum grain (CSR01) were sorted to remove stones, broken grains, non uniform sized grains and other foreign materials. 300g of the sorted grains was washed in a clean bucket and soaked in $900cm^3$ of deionized water containing 0.1% formaldehyde to inhibit microbial growth. Steeping was done for 24 hours with 2 hours air rest between 11th to 13th hours of steeping (i.e. 12 hours water steep, 2 hours air rest and 10 hours water steep in a fresh $900cm^3$ of deionized water containing 0.1% formaldehyde). The steeped grains were allowed to germinate in dark chamber for 5 days, with intermittent turning and spraying of $30cm^3$ of deionized water 12 hourly to avoid matting and drying up.

Germination was stopped by drying the germinated grains in an oven at a temperature of 55^{o}C for 24 hours (kilning). The kilned grains were derooted or deculmed by rubbing in an undulated surface to separate the rootlets. The 'malt' was then weighed and the percentage malting loss-determined as follows:

$Malting\ Loss$: $\frac{W_{bm}-W_{am}}{W_{bm}} \times \frac{100}{1}$

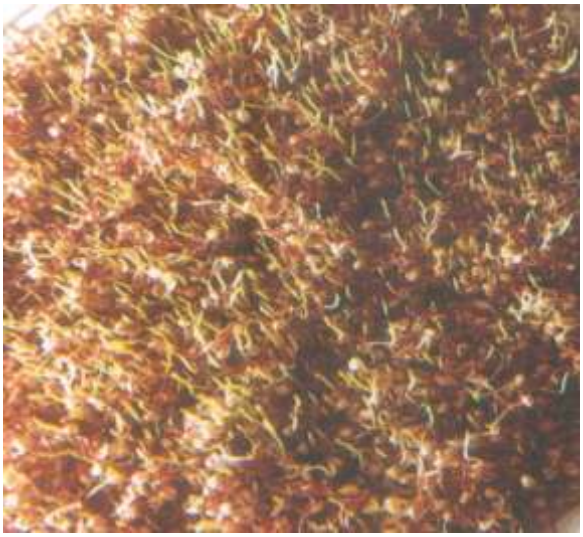
where

W_{bm} = Weight of grain before malting

W_{am} = Weight of grain after malting for a given number of germination days.

Mashing: 200g of malted grains was milled to coarse particles using a laboratory milling machine (Gibbons, Model 8). The milled malt was mixed with water (800ml) at 40^{o}C in a regulated water bath and allowed to stand for 30 minutes with intermittent stirring. The temperature of the mash was increased to 55^{o}C and the time taken for the mash to reach 55^{o}C was noted. The mash was allowed to rest at 55^{o}C for 30minutes, with stirring every 10 minutes. The temperature of the mash was further raised to 65^{o}C and the time it took was recorded. The mash was again allowed to rest at 65^{o}C for 30 minutes, with stirring every 10 minutes. The temperature was then raised to 72^{o}C, noting the time taken to reach this temperature and allowed to stand for 10 minutes. The temperature was reduced to 60^{o}C and the time taken for the temperature of the mash to come down to 60^{o}C was recorded.

(a)



(b)

(c)

(d)

(e)

(f)

Fig. 3.1: Processes involved in brewing (a) Sorghum grains (CSR01) before sorting (b) CSR01 after 5 days of germination (c) CSR01 after kilning (d) removed rootlets (e) mashing regime (f) fermentation.

At this temperature, 5cm^3 of exogenous enzyme (α-amylase-fungamyl) was added and allowed to rest at this temperature for 30 minutes. The temperature was then increased to 75^{o}C noting the time it took to reach 75^{o}C and heated for 10 minutes to denature the enzyme. The mash was filtered into 500cm^3 conical flask using Whatman No. 1 filter paper. The volume of the wort recovered was measured.

The wort was boiled for 20 minutes and the final volume recorded.

Fermentation: The wort was divided into 6 portions and cooled to 8 – 10^{o}C. A 5g of brewer's yeast, a bottom-fermenting type (*Saccharomyces uvarum*) was inoculated into 100ml of the 6 wort portions in 250cm^3 fermenting flasks for primary fermentation for 5 days at 8 – 10^{o}C observing for yeast flocculation. The fermented 'green beer' was carefully decanted into another set of 250cm^3 fermenting flasks. 0.1ml each of isomerized hop extract, extracts of hop leaf, *G. kola, A. indica*, *V. amygdalina* and *G. latifolium* was added to the 6 green beer portions in the fermenting flasks labeled A, B, C, D, E and F respectively. The flasks and contents were kept in a refrigerator at 8 – 10^{o}C for secondary fermentation /maturation for twenty one days. Final beer filtration was carried out and the 'matured' beer samples transferred to sample bottles for analysis.The sample bottles that contained the respective beers were labeled accordingly.

3.2.9 Physicochemical Properties of Beer

Alcohol Content

Distillation method as described by Ceiwryn (1999) was employed. A 50ml sample was measured into 150 ml volumetric flask at 20^{o}C and washed into a distillation flask using 100ml of water. The solution was neutralized with 2ml of 1M NaOH solution. The solution was distilled and the distillate collected. The distillate was cooled to 20^{o}C and the specific gravity calculated. The alcohol strength was subsequently determined by reference to Alcoholometric Table (Appendix 3).

Calculation:

$$\text{Specific gravity} = \frac{X_2 - X_1}{X_3 - X_1}$$

where

X_1 = Weight of empty specific gravity bottle

X_2 = Weight of specific gravity bottle + sample

X_3 = Weight of specific gravity bottle + water

Total Acidity

A 25ml sample was boiled under reflux for 20 minutes to expel CO_2. The condenser was washed down with deionized water to make up to the original volume of the sample (25ml). The resulting solution was titrated with 0.1M NaOH solution using bromothymol blue as indicator.

$$\text{Percentage total acidity} = \frac{\text{Titre value} \times \text{Factor equivalent}}{\text{Volume of sample}} \qquad \text{(Ceiwryn, 1999)}$$

Factor equivalent of acetic acid = 0.006

pH

pH was measured by Electrometric method using laboratory pH meter as described by Food Compliance Laboratory Unit of National Agency for Food and Drug Administration and Control (NAFDAC SOP Code: FC:06.5). The electrodes were rinsed with distilled water and blot dried. The pH electrode was then rinsed in a small beaker with a portion of the sample. Sufficient amount of the sample was poured into a small beaker to allow the tips of the electrodes to be immersed to a depth of about 2cm. The electrode was at least 1cm away from the sides and bottom of the beaker. The temperature adjustment vial was adjusted accordingly. The pH meter was turned on and the pH of the sample recorded.

Turbidity

AOAC method 970.14 was selected. A 2ml sample was placed in a clean, dry turbidity vial and capped securely. Excess liquid or fingerprint was wiped off with a soft cloth. The vial and content was placed into the AQ4500 sample chamber. The measure key was then pressed and the result displayed on the instrument with NTU.

NTU= Nephelometric Turbidity Unit.

Total Solids

The method employed was as detailed by Food compliance laboratory unit of National Agency for Food and Drug Administration and Control (NAFDAC), Oshodi, Lagos (NAFDAC SOP Code: FC: 07.2.2). The Satorious moisture analyzer was switched on from the mains till off is displayed on the dash board. The (1/Q) ON/OFF key was pressed to display the satorious logo followed by the 0.000g and the heating programmer. The instrument was allowed to warm up and stabilize for at least 30 minutes in order to reach 105^{o}C. The sample chamber was opened to position a new sample pan draft shield on the pan support. The sample pan was tarred (zeroed) by selecting the tare function as needed to get 0.000g on the dash board and the ENTER key pressed. The prepared sample was weighed and spread on the pan draft shield uniformly. The sample chamber was closed and the drying programmer started when the ENTER key was pressed. The drying programmer shuts off automatically once no further moisture or weight loss is detected.

The percentage weight loss due to the amount of moisture loss is displayed automatically and the percentage moisture loss is recorded.

Total Solids = 100 – % moisture loss.

Micro metals (Arsenic, Cadmium and Copper)

Before the analysis, all beer samples were degassed using an ultrasonic bath for 30 minutes. A 10ml aliquot of the degassed sample was mixed with 2ml of

concentrated nitric acid and 2ml of hydrogen peroxide in a digestion tube. The mixture was heated for 1 hour at 100^{o}C until complete clarification and allowed to cool, and filtered and diluted to 25ml with deionized water. Analytical blanks were prepared in a similar manner, but omitting the test sample. The solutions were subsequently analyzed for Arsenic, Cadmium and Copper using Atomic Absorption Spectrophotometer.

Bitterness Level

Bitterness was determined according to ASBC Beer 23A. Ten mililitre of decarbonated and foam freed beer sample was measured into a 50ml centrifuge tube. To this was added 1ml 3M hydrochloric acid and 20ml iso-octane. The tube was stoppered and agitated for 15 minutes. The tube was further centrifuged for 3 minutes at 3000 rpm.

An iso-octane blank was prepared into a 1cm quartz curvet. A clear iso-octane phase in the centrifuge tube was pipetted into another curvet and stoppered.

The spectrophotometer's λ was set at 275nanometer and zeroed with the blank before the absorbance of the sample was read. The reading was multiplied by 50 and the result expressed as:

Absorbance at 275 nanometer x 50 = Bitterness in IBUwhere IBU = International Bitterness Unit.

3.2.10 Statistical Analysis

Tables were generated using the Schimadzu GC – MS solution software and MS library. From triplicate experiments in phytochemical screening and metal content analysis of extracts, and the investigation of physicochemical properties of the brewed beer samples, the mean, standard deviation and range in all the studies were evaluated (Appendix 4). The mean values were used to construct bar charts for easy interpretation of results.

Simple statistics (ranking) was employed to determine the significant difference between the controls (hops) and the Nigerian vegetables. In ranking, we determined the existence of the significant difference among isomerized hop (control), *G. kola, A. indica, V. amyadalina* and *G. Latifolium* on one hand and hop leaf (control), *G. kola, A. indica, V. amygdalina* and *G. latifolium* on the other hand. We employed test of significant difference using one way Analysis of Variance (ANOVA). The software used for the analysis was SPSS (Special Package for Social Sciences) Version 20.

3.2.11 Ranking

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). 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. The results/outputs of this test using SPSS (Special Package for Social Science) version 20 are annexed as Appendix 5.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 EXTRACT QUALITY

4.1.1 The Extracts

Fig. 4.1 (a-f) shows isomerized hop extract and the extracts from hop leaf, *G. kola*, *A. indica*, *V. amygdalina* and *G. latifolium*. All the extracts were obtained as viscous syrup.

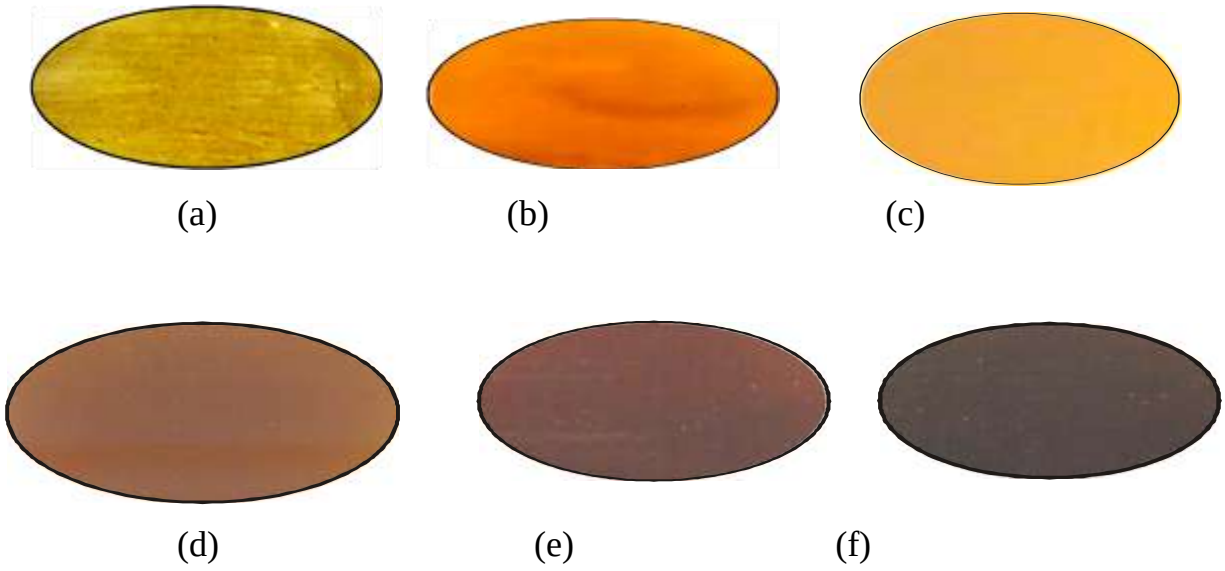


Fig 4.1: The extracts (a) Isomerized hop (b) Hop leaf (c) *G. kola* (d) *A. indica* (e) *V. amygdalina* (f) *G. latifolium*

4.1.2 TLC Results

The extracts of *G. kola, A. indica, V. amygdalina, G. latifolium,* hop leaf and isomerized hop extract showed a total number of 12, 10, 9, 10, 11 and 14 fractions respectively in the TLC chromatograms. This result was further confirmed by the GC chromatograms in Appendix 2.

4.2 CHEMICAL CONSTITUENTS OF THE EXTRACTS

4.2.1 *Garcinia kola* (Bitter cola):

The extract of *G. kola* contained twelve constituents (Appendix 2A) and these constituents are shown in Table 4.1. It is shown in the Table that 6-octadecenoic acid was highest with a proportion of 44.09 %.

Table 4.1: Relative Proportion of Constituents of *G. kola*

S/N	Constituent	Formula	Relative Proportion (%)
1.	Hexadecanoic, methyl ester	$C_{17}H_{34}O_2$	0.69
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	9.30
3.	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	2.84
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	1.62
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	44.09
6.	Octadecanoic acid	$C_{18}H_{36}O_2$	23.31
7.	2-Methyl-3, 13-octadecadein-1-ol	$C_{19}H_{36}O$	4.23
8.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$	1.04
9.	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$	1.92
10.	9-Hexadecenal	$C_{16}H_{30}O$	7.07
11.	Octadecanoic acid, 2-hydroxyl-1, 3-propanediyl ester	$C_{39}H_{76}O_5$	2.84
12.	Hexadecanoic acid, 2, 3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	1.04

There was also presence of 9, 12-octadecadienoic acid, the grape seed oil which is an essential oil of the hops. Hexadecanoic acid was least in proportion with 0.69 %.

4.2.2 *Azadirachta indica* (Neem)

A. indica extract contained ten (10) constituents as shown in Appendix 2B. Table 4.2 shows these constituents and their relative proportion. It is shown in the Table that 6-octadecenoic acid had the highest proportion of 44.96 % and hexadecanoic acid- 2, 3-dihydroxypropyl ester had the least proportion of 0.85 %.

Table 4.2: Relative Proportion of Constituents of *A. indica*

S/N	Constituent	Formula	Relative Proportion (%)
1.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	1.41
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	9.57
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	4.34
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	2.36
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	44.96
6.	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	24.58
7.	Hexadecanoic acid, 2-hydroxyl-1, 3-propanediyl ester	$C_{35}H_{68}O_5$	1.95
8.	9, 12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$	7.15
9.	Octadecanoic acid, oxiranylmethyl ester	$C_{21}H_{40}O_3$	2.83
10.	Hexadecanoic acid, 2, 3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	0.85

The grape seed oil, an essential oil of the hop cone was present in this sample with a proportion of 7.15 % which is higher than the proportion of the grape seed oil in both isomerized hop extract and the hop leaf extract as shown in Table 4.7. There were also present of other constituents in this extract which were absent in

imported hops that may possibly give hop characters to beer. Based on these observations, *A. indica* could be a substitute for hops in beer brewing.

4.2.3 *Vernonia amygdalina* (Bitter Leaf)

The extract of *V. amygdalina* contained the least number of constituents. Appendix 2C shows that this sample contained only nine (9) constituents. These constituents and their relative proportion are shown in Table 4.3. It is shown in the Table that 6 – octadecenoic acid had the highest proportion of 43.42 % just like it had in isomerized hop extract and hop leaf extract as shown in Table 4.7. Based on this alone, *V. amygdalina* could substitute imported hops in beer brewing. Incidentally, this sample did not contain the essential oil of hop, the grape seed oil (9, 12-octadecadienoic acid). If this constituent is not neglected, then *V. amygdalina* cannot substitute hops in beer brewing; although this extract contained other constituents common to the isomerized hop and hop leaf such as 11-octadecanoic acid methyl ester ($C_{19}H_{36}O_2$), octadecanoic acid methyl ester ($C_{19}H_{38}O_2$) and hexadecanoic acid ($C_{16}H_{32}O_2$) as presented in Table 4.7.

Table4.3: Relative Proportion of Constituents of *V. amygdalina*

S/N	Constituent	Formula	Relative Proportion (%)
1.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	1.46
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	9.23
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	5.12
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	2.63
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	43.42
6.	Octadecanoic acid	$C_{18}H_{36}O_2$	24.06
7.	Hexadecanoic acid, 2-hydroxyl-1, 3-propanediyl ester	$C_{35}H_{68}O_5$	2.25
8.	9-Hexadecenal	$C_{16}H_{30}O$	8.31
9.	Octadecanoic acid, oxiranyl methyl ester	$C_{21}H_{40}O_3$	3.51

All these constituents may contribute to the hop character of beer when *V. amygdalina* is used in lieu of imported hops.

4.2.4 *Gongronema latifolium* (Heckel)

From Appendix 2D, it is shown that the extract of *Gongronema latifolium* contained 18 constituents. These constituents and their relative proportion are shown in Table 4.4. This extract contained both 6-octadecenoic acid and 9,12-octadecadienoic acid (grape seed oil) in proportions of 44.6 % and 7.95 % respectively. The Table shows that this extract contained other constituents such as hexadecanoic acid, 11-octadecenoic acid methyl ester, octadecanoic acid methyl ester, and octadecanoic acid 2-hydroxyl-1, 3-propanediyl ester ($C_{39}H_{76}O_5$). Extracts of imported hops also contained the aforementioned constituents (Table 4.7).

Table 4.4: Relative Proportion of Constituents of *G. latifolium*

S/N	Constituent	Formula	Relative Proportion (%)
1.	Benzoic acid, 2-(aminocarbonyl)	$C_8H_7NO_3$	2.89
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	10.69
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.77
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	0.65
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	44.61
6.	Octadecanoic acid, 2-(2-hydroxyl-ethoxy) ethyl ester (*Aqua cera*)	$C_{22}H_{44}O_2$	25.46
7.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$	7.95
8.	9,12-Octadecadien-1-ol	$C_{18}H_{34}O$	4.91
9.	9-Hexadecenal	$C_{16}H_{30}O$	1.46
10.	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{16}O_5$	0.61

Based on these observations, *G. latifolium* could be a possible substitute to imported hops in beer brewing. Although the derivative of the alpha acid (dehydrocohumulunic acid) was conspicuously absent in this sample as sown in Table 4.7, there are other constituents which were present in *G. latifolium* but were absent in imported hops. Such constituents include benzoic acid-2-(amino carbonyl), and octadecanoic acid, 2-(2-hydroxylethoxy) ethyl ester, *aqua cera*.

4.2.5 Hop leaf

This sample contained eleven constituents (Appendix 2E) with 6 – octadecenoic acid having the highest proportion of 43.55 % and hexadecanoic acid, methyl ester having the least proportion of 1.13 %.

Table 4.5 shows these constituents and their relative proportion. There was presence of lupulone (beta-lupulic acid) in hop leaf extract. Lupulone is a β-acid which contributes marginally in bitterness of beer. Also present in this sample was the grape seed oil. This is an essential oil of the hop cone responsible for flavour and aroma enhancement in finished beer. All the other constituents present in this extract may contribute to other hop characters in beer.

Table 4.5: Relative Proportion of Constituents of Hop leaf

S/N	Constituent	Formula	Relative Proportion (%)
1.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	1.13
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$	9.54
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	3.14
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	2.45
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	43.55
6.	Octadecanoic acid	$C_{18}H_{36}O_2$	25.56
7.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$	1.26
8.	Octadecanoic acid, 2-hydroxyl-1,3-propanediyl ester	$C_{39}H_{76}O_5$	1.63
9.	Lupulone (beta-lupulic acid)	$C_{26}H_{38}O_4$	2.02
10.	9-Hexadecenal	$C_{16}H_{30}O$	5.59
11.	Octadecanoic acid, oxiranyl, methyl ester	$C_{21}H_{40}O_3$	4.13

4.2.6 Isomerized hop

Isomerized hop extract contained fourteen constituents as shown in Appendix 2F with 6-octadecenoic acid having the highest proportion of 28.92%. The constituents and their relative proportion are shown in Table 4.6.

Table 4.6: Relative Proportion of Constituents of Isomerized hop

S/N	Constituent	Formula	Relative Proportion (%)
1.	4,4-Dimethyl-2-buten-4-olide	$C_6H_8O_2$	3.62
2.	1,2-Dimethl cyclopropane carboxylic acid	$C_6H_{10}O_2$	9.90
3.	2,5-Dimethyl-2-hexanol	$C_8H_{18}O$	2.68
4.	Dehydro-cohumulinic acid	$C_{14}H_{18}O_3$	5.33
5.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	7.84
6.	4,4,5,5-Tetramethyl-bicyclo-hexyl-6-ene-2,3 dione	$C_{16}H_{24}O_2$	9.25
7.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	3.69
8.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	1.21
9.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	28.96
10.	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	17.92
11.	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$	1.24
12.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$	4.65
13.	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	2.57
14.	1,2-Benzendicarboxylic acid, bis (2-ethylhexyl) ester	$C_{24}H_{38}O_4$	1.14

There was presence of dehydro-cohumulunic acid, a derivative of an alpha acid called cohumulone.Cohumulone generates isocohumulone by isomerization. Isocohumulones are chemical compounds that contribute to the bitter taste of beer and are in the class of compounds known as iso-alpha acids which contain approximately 40-80% bitter principles in the hop resin. The hop resin is known for its characteristic bitter taste in beer.

There was also the presence of 9,12-octadecadienoic acid, the grape seed oil, which is an essential oil of the female hop cone responsible for flavouring and aroma enhancement in beer. All the other constituents are however responsible for other characters of hop e.g. pronounced bacteriostatic activity that inhibits the

growth of gram-positive bacteria in the finished beer and precipitation of proteins.

4.2.7 Constituents Comparison of all the Extracts

Table 4.7 shows constituents of the extracts from Nigerian plants in comparison with extracts of isomerized hop and hop leaf.

It is evident from Table 4.7 that 4,4-dimethyl-2-buten-4-olide ($C_6H_8O_2$); 1,2-dimethyl cyclopropane carboxylic acid ($C_6H_{10}O_2$); 2,5-dimethyl-2-hexanol ($C_8H_{18}O$); 4,4,5,5-tetramethyl bicyclo hexyl-6-ene-2,3,-dione ($C_{16}H_{24}O_2$); 1,2-benzen dicarboxylic acid bis (2-ethyl hexyl) ester ($C_{24}H_{38}O_4$) and dehydro-cohumulunic acid ($C_{14}H_{18}O_3$) are present in isomerized hop extract only.

It is also observed that hop leaf extract only contained lupulone ($C_{26}H_{30}O_4$), a β-acid known as beta-lupulic acid and octadecanoic acid oxiranyl methylester ($C_{21}H_{46}O_3$). All the extracts contained hexadecanoic acid ($C_{16}H_{32}O_2$), octadecenoic acid methyl ester ($C_{19}H_{36}O_2$), octadecanoic acid methyl ester ($C_{19}H_{38}O_2$) and 6-octadecenoic acid ($C_{18}H_{34}O_2$).

The extracts of hop leaf, isomerized hop, *G. kola, A. indica* and *V. amygdalina* contained octadecanoic acid ($C_{18}H_{36}O_2$) in common while the extracts of isomerized hop, hop leaf, *G. kola, A. indica* and *G. latifolium* contained 9, 12-octadecadienoic acid, the grape seed oil ($C_{18}H_{32}O_2$) in common. The extracts of isomerized hop, hop leaf, *G. kola*, and *G. latifolium* only contained octadecanoic acid, 2-hydroxyl-1, 3-propandiyl ester ($C_{39}H_{76}O_5$).

Another significant observation is that each of the extracts of hop leaf, *G. kola, V. amygdalina* and *G. latifolium* contained 9-hexadecenal ($C_{16}H_{30}O$) which was absent in extracts of isomerized hop and *A. indica.* Also, each of the extracts of hop leaf, *G. kola*, *A. indica* and *V. amygdalina* contained hexadecanoic acid methyl ester ($C_{17}H_{34}O_2$). Hexadecanoic acid methyl ester was not present in both the extracts of isomerized hop and *G. latifolium*.

However, there are constituents 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-octadecadien-1-ol ($C_{19}H_{36}O$), *G. latifolium* alone contained octadecanoic acid -2-(2-hydroxyethoxy) ethylester ($C_{22}H_{44}O_2$) which is *aqua cera* and 9, 12-octadecadiene-1-ol ($C_{18}H_{34}O$). All these constituents were completely absent in imported hops. This minor differences and major similarities in the constitution of chemical constituents in the local plants 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 4.7 Constituents comparison of all the Extracts

S/N	Constituet	Isomerized hop	Hop leaf	*G. kola*	*A. indica*	*V. amygdalina*	*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	9.30	9.57	9.23	10.69
8.	Octadecenoic acid, methyl ester	3.69	3.14	2.84	4.34	5.12	0.77
9.	Octadecanoic acid, methyl ester	1.21	2.45	1.62	2.36	2.63	0.65
10.	6-octadecenoic acid	28.96	43.55	44.09	44.96	43.42	44.61
11.	Octadecanoic acid	17.92	25.56	23.31	24.58	24.06	-
12.	Hexadecanoic acid, 2-hydroxy -1,3-propanediyl ester	1.24	-	1.92	1.95	2.25	-
13.	9,12-octadecadienoic acid (grape seed oil)	4.65	1.26	1.04	7.15	-	7.95
14.	Octadecanoic acid, 2-hydroxyl -1,3-propanediyl ester	2.57	1.63	2.84	-	-	0.61
15.	Hexadecanoic acid, methyl ester	-	1.13	0.69	1.41	1.46	-
16.	Lupulon (beta-lupulic acid)	-	2.02	-	-	-	-
17.	Octadecanoic acid, oxiranyl methyl ester	-	4.13	-	2.83	3.51	-
18.	9-hexadecenal	-	5.59	7.07	-	8.31	1.46
19.	2-methyl-3,13-octadecadienol	-	-	4.23	-	-	-
20	Octadecanoic acid, 2(-2-hydroxyethoxy) ethylester	-	-	-	-	-	25.46
21	9,12-octadecadien-1-ol	-	-	-	-	-	4.91
22.	Benzoic acid, 2(aminocarbonyl)	-	-	-	-	-	2.89
23.	Hexadecanoic acid-2,3-dihydroxypropyl ester	-	-	1.04	0.85	-	-

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.

4.3 PHYTOCHEMICALS IN THE EXTRACTS

Phytochemicals investigated in this study include: alkaloids, tannins, saponins, oxalates, phytates, trypsin inhibitors, cardiac glycosides, cyanogenic glycosides and hydrogen cyanide.

4.3.1 Alkaloids

The alkaloid contents of the samples studied are shown in Table 4.8 and Fig. 4.2. 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 4.8: Alkaloid content in the samples

Sample	Alkaloid (%)			
	Mean	St Dev	Range	
Isomerized hop	3.2	0.265	0.5	p_1=0.633>0.05
Hop leaf	4.0	0.300	0.6	p_2=0.978>0.05
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	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

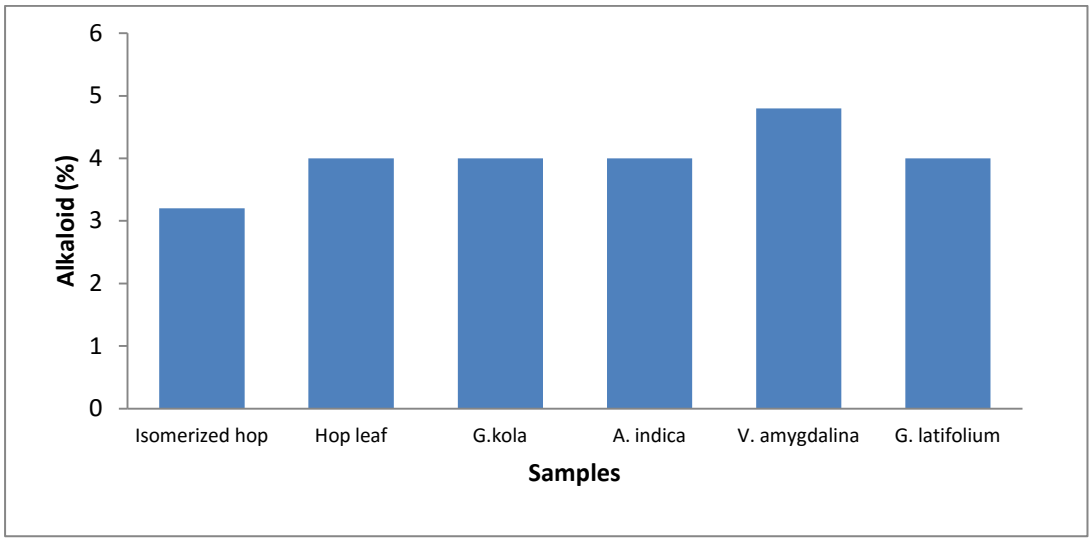


Fig. 4.2: Alkaloid content in the samples.

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).

4.3.2 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 4.9 and Fig. 4.3 show 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 4.9: Tannin content in the samples.

Sample	Tannin (%)			
	Mean	**St Dev**	**Range**	
Isomerized hop	3.6	0.300	0.6	p_1=0.633>0.05
Hop leaf	2.0	0.173	0.3	p_2=0.978>0.05
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	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

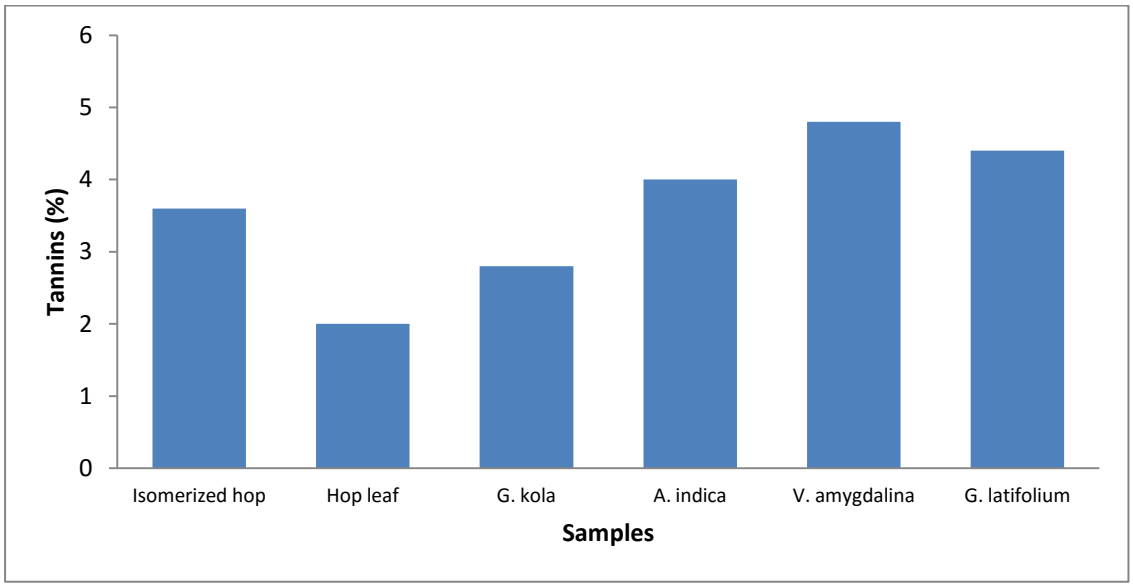


Fig. 4.3: Tannin content in the samples.

Tannins (commonly referred to as tannic acids) are polyphenols present in many plant foods that form colloidal solution in water (Buttler and Bailey, 1973).

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) 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).

4.3.3 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 4.10 and Fig. 4.4).

Table 4.10: Saponin content in the samples

Sample	Saponin (%)			
	Mean	St Dev	Range	
Isomerized hop	2.8	0.458	0.9	p_1=0.633>0.05
Hop leaf	3.2	0.346	0.6	p_2=0.978>0.05
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	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

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.

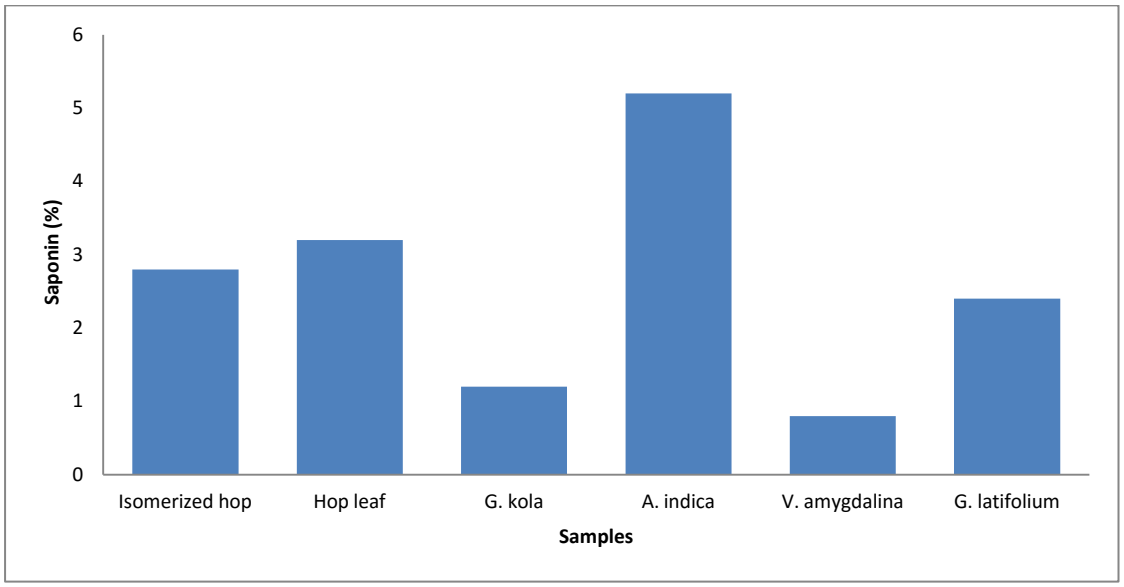


Fig. 4.4: Saponin content in the samples.

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).

4.3.4 Oxalates

Oxalate, a conjugate base of the oxalic acid ($H_2C_2O_4$), is a naturally occurring substance found in plants and animals. It 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 (Curhan, 1999).

Table 4.11: Concentration of oxalate in the samples

Sample	Concentration (mg/100g)			
	Mean	**St Dev**	**Range**	
Isomerized hop	0.0405	0.00934	0.0185	p_1=0.633>0.05
Hop leaf	0.0432	0.00735	0.0146	p_2=0.978>0.05
G.kola	0.10200	0.00260	0.0046	
A. indica	0.0540	0.00409	0.0080	
V. amygdalina	0.0486	0.00791	0.0157	
G. latifolium	0.0540	0.00368	0.0071	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

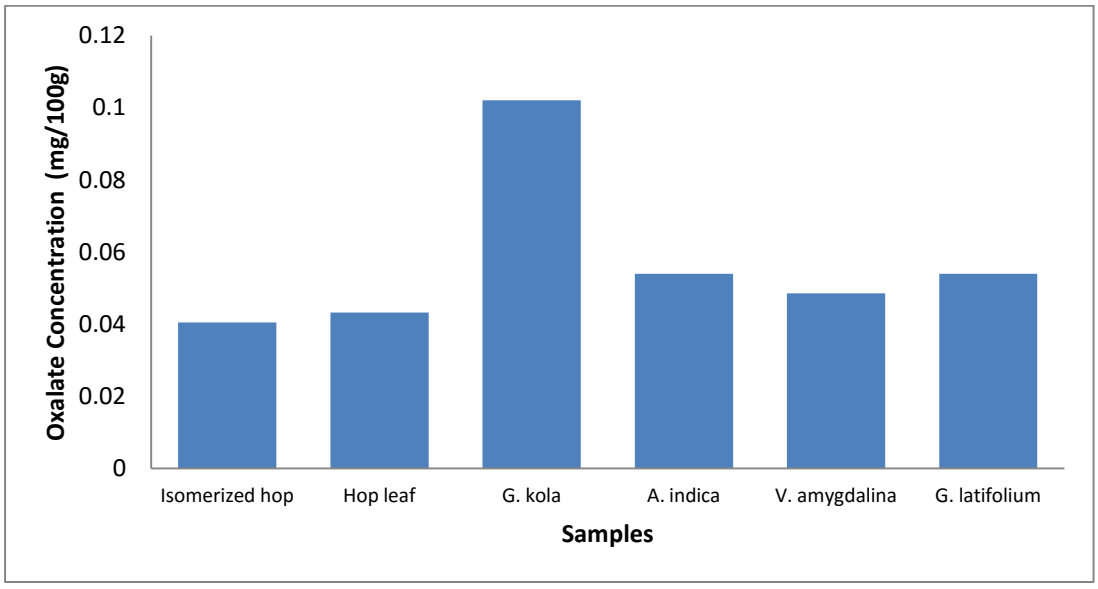


Fig. 4.5: Concentration of oxalate in the samples.

Close examination of Table 4.11 and Fig. 4.5 show that Oxalate concentration was high in *G. kola* but comparatively close in all the other samples. Therefore, *A. indica*, *V. amygdalina* and *G. latifolium* can substitute hops

4.3.5 Phytatic acid

Phytic acid was virtually of the same percentage range in all the samples especially hop leaf, *V. amygdalina* and *G. latifolium*. *G. kola* and *A. indica* did not differ much in their percentage content of phytic acid while isomerized hop was moderate in comparison with the others. Table 4.12 and Fig. 4.6 show these relationships among the samples. 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.

Table 4.12: Phytic acid content in the samples

Sample	**Phytic acid (%)**			
	Mean	**St Dev**	**Range**	
Isomerized hop	1.39	0.0173	0.03	p_1=0.633>0.05
Hop leaf	1.68	0.0624	0.12	p_2=0.978>0.05
G.kola	0.99	0.0361	0.07	
A. indica	1.16	0.0608	0.11	
V. amygdalina	1.62	0.1253	0.25	
G. latifolium	1.51	0.1820	0.32	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

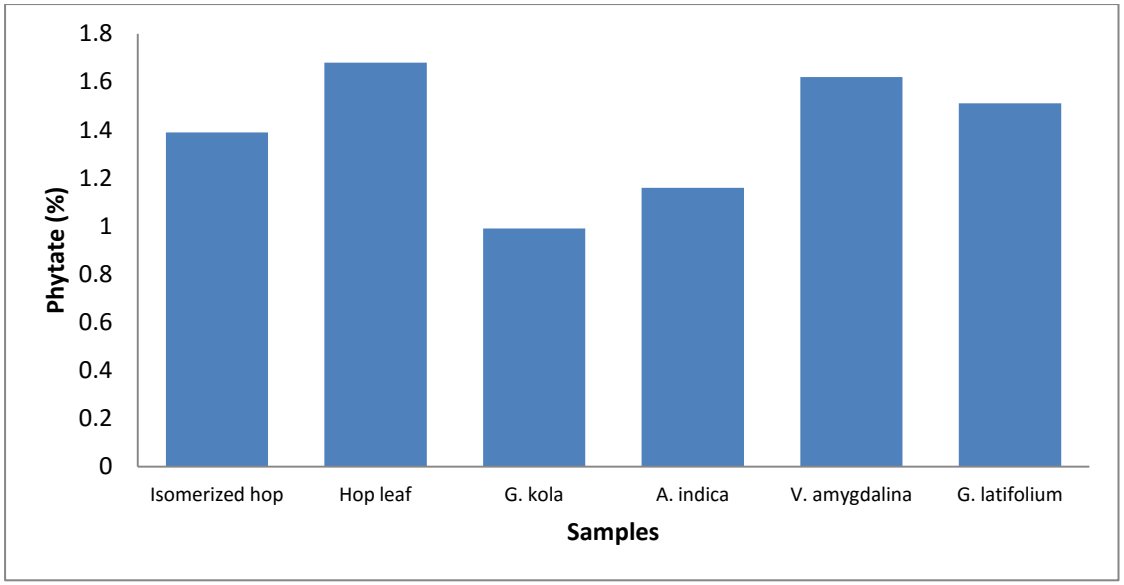


Fig. 4.6: Phytic acid content in the samples.

Phytic acid, also known as inositol hexaphosphate (IP6), or phytate 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 (Edman and Forbes, 1977). On the other hand, those authors reported that phytic acid is a powerful antioxidant as well as helpful in ridding the body of heavy metals and other toxins.

4.3.6 Trypsin Inhibitors

Trypsin inhibitors are chemicals that reduce the availability of biological active trypsin, an enzyme essential to nutrition of many animals, including humans. The trypsin inhibitors are reported to be one of the major toxic components of legumes (Liener and Kakade, 1980).

Table 4.13: Trypsin inhibitors in the samples

Sample	Trypsin inhibitors (%)			
	Mean	St Dev	Range	
Isomerized hop	6.45	0.427	0.85	p_1=0.633>0.05
Hop leaf	7.60	0.361	0.70	p_2=0.978>0.05
G.kola	2.80	0.608	1.10	
A. indica	9.60	0.600	1.20	
V. amygdalina	16.45	1.262	2.50	
G. latifolium	17.30	2.270	4.20	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plans

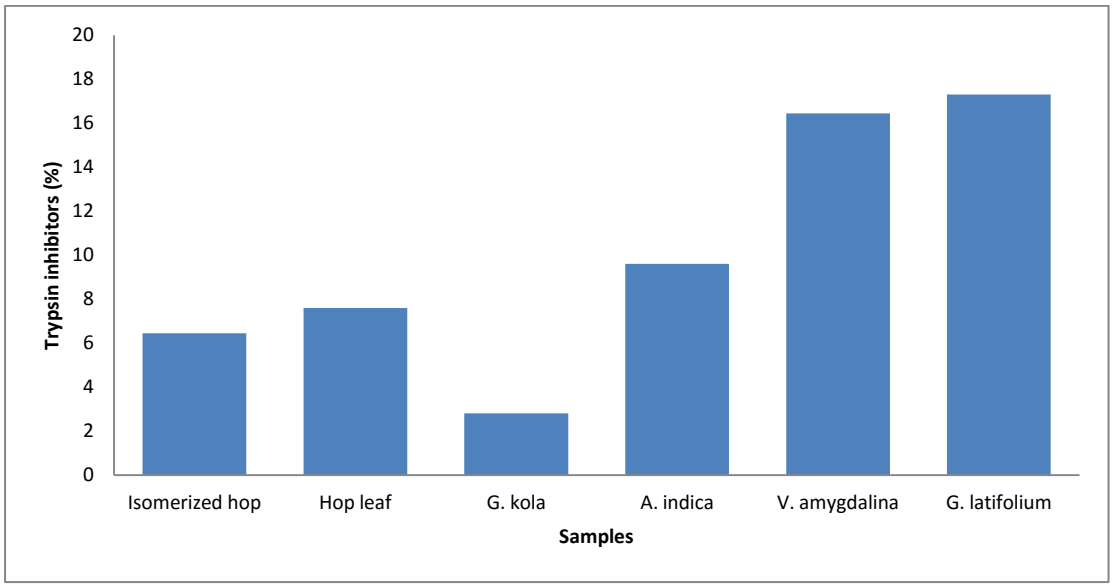


Fig. 4.7: Trypsin inhibitors in the samples.

The percentage trypsin inhibitor units was 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* (Table 4.13 and Fig. 4.7). 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.

4.3.7 Haemagglutinins

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 4.14 and Fig. 4.8.

Table 4.14: Concentration of Haemagglutinin in the samples

Sample	**Concentration (mg/g)**			
	Mean	**St Dev**	**Range**	
Isomerized hop	6.372	1.407	2.777	p_1=0.633>0.05
Hop leaf	6.669	0.584	1.113	p_2=0.978>0.05
G.kola	3.879	0.875	1.724	
A. indica	7.270	0.465	0.819	
V. amygdalina	6.654	0.399	0.766	
G. latifolium	6.672	0.809	1.547	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

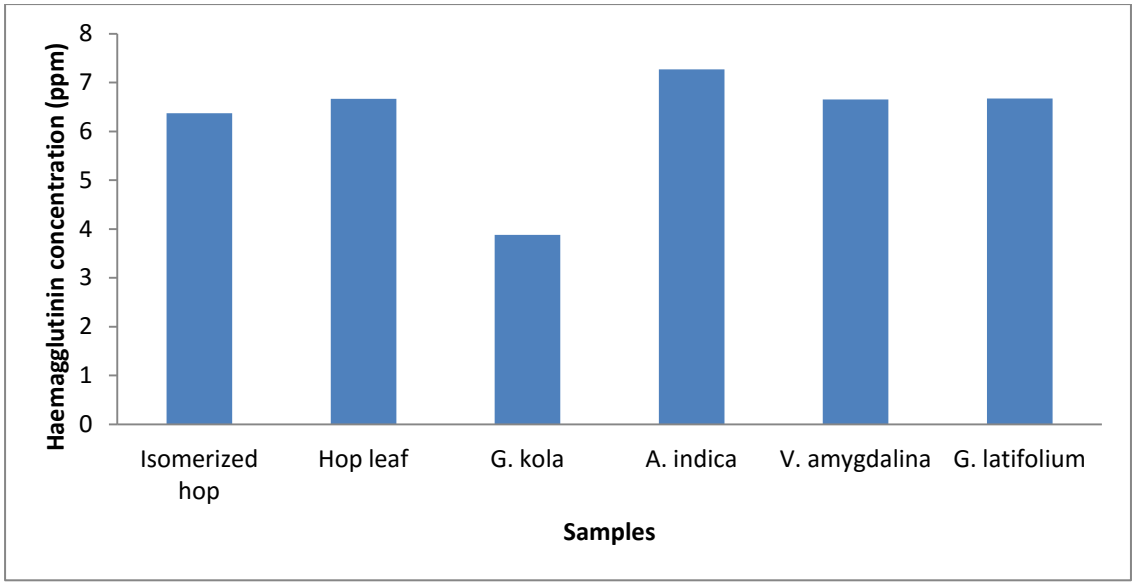


Fig. 4.8: Concentration of haemagglutinin in the samples.

Haemagglutinin refers to a substance that causes red blood cells to agglutinate, a process known as haemagglutination. Antibodies (Russel *et al*., 2000) and lectin (Nelson and Cox, 2005) are common known haemagglutinins.

4.3.8 Cardiac glycosides

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. These glycosides are found as secondary metabolites in several plants (Wang *et al*., 2008).

From this study, it is evident that cardiac glycosides were available in all the samples (Table 4.15 and Fig. 4.9). 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.

Table 4.15: Cardiac glycoside in the samples

Sample	Cardiac glycoside (%)			
	Mean	**St Dev**	**Range**	
Isomerized hop	4.5	0.917	1.8	p_1=0.633>0.05
Hop leaf	3.5	0.624	1.2	p_2=0.978>0.05
G.kola	4.0	0.850	1.7	
A. indica	6.0	0.346	0.6	
V. amygdalina	5.0	0.954	1.9	
G. latifolium	5.5	0.400	0.8	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

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.

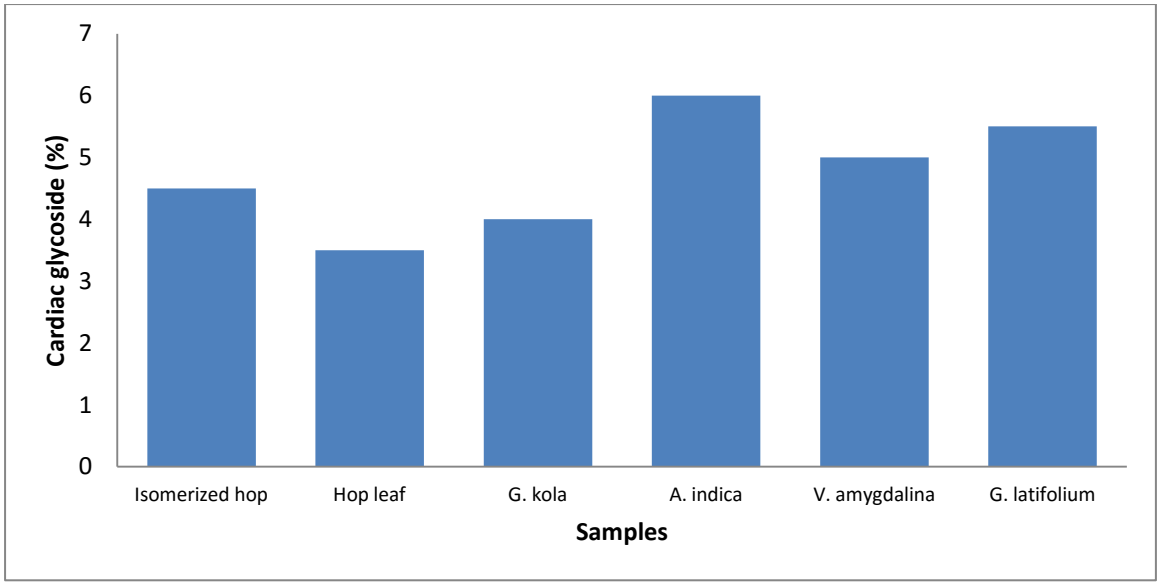


Fig. 4.9: Cardiac glycoside in the samples.

Moreover, therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure, congestive heart failure, and as heart tonics, diuretics and emetics (Wang *et al*., 2008).

4.3.9 Cyanogenic glycoside

The concentration of cyanoglycoside was relatively low in *A. indica.* Table 4.16 and Fig. 4.10 show that the concentrations of cyanogenic glycosides in isomerized hop and *V. amygdalina* did not differ much and those of hop leaf, *G. kola* and *G. latifolium* were comparatively uniform. Thus, except *G. kola*, all the other samples could substitute one another.

Table 4.16: Concentration of Cyanogenic glycoside in the samples

Sample	Concentration (ppm)			
	Mean	St Dev	Range	
Isomerized hop	0.648	0.0451	0.08	p_1=0.633>0.05
Hop leaf	0.810	0.1000	0.20	p_2=0.978>0.05
G.kola	0.756	0.0490	0.091	
A. indica	0.216	0.0779	0.153	
V. amygdalina	0.594	0.0896	0.173	
G. latifolium	0.702	0.0744	0.138	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

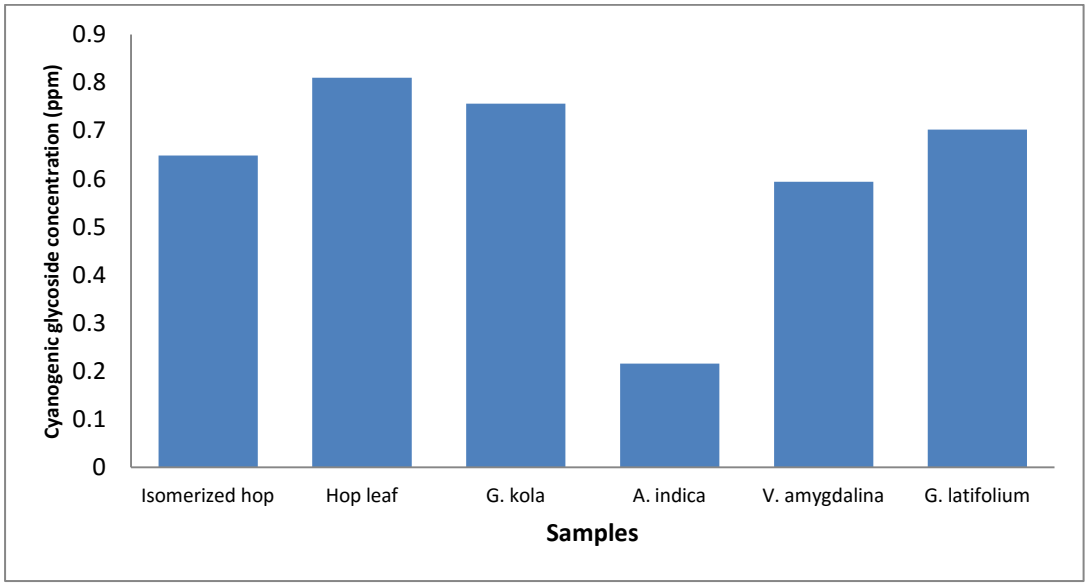


Fig. 4.10: Concentration of Cyanogenic glycoside in the samples.

Cyanogenic glycosides or cyanoglycosides account for approximately 90% of the wider group plant toxins known as cyanogens (Agba-Egbe and Lape, 2006). Potential toxicity of cyanoglycosides arises from enzymatic degradation to

produce free hydrogen cyanide (cyanogenesis), resulting in acute cyanide poisoning. 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 (Davis, 1991; Haque 2002; Simeonova and Fishbein, 2004). 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.

4.3.10 Hydrogen cyanide

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 also a good substitute to Hop leaf (Table 4.17 and Fig. 4.11). 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.

Table 4.17: Concentration of Hydrogen cyanide in the samples

Sample	**Concentration (ppm)**			
	Mean	**St Dev**	**Range**	
Isomerized hop	0.756	0.0674	0.133	p_1=0.633>0.05
Hop leaf	0.648	0.1046	0.192	p_2=0.978>0.05
G.kola	0.648	0.1034	0.206	
A. indica	0.540	0.1201	0.230	
V. amygdalina	0.756	0.0527	0.104	
G. latifolium	1.404	0.4210	0.790	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

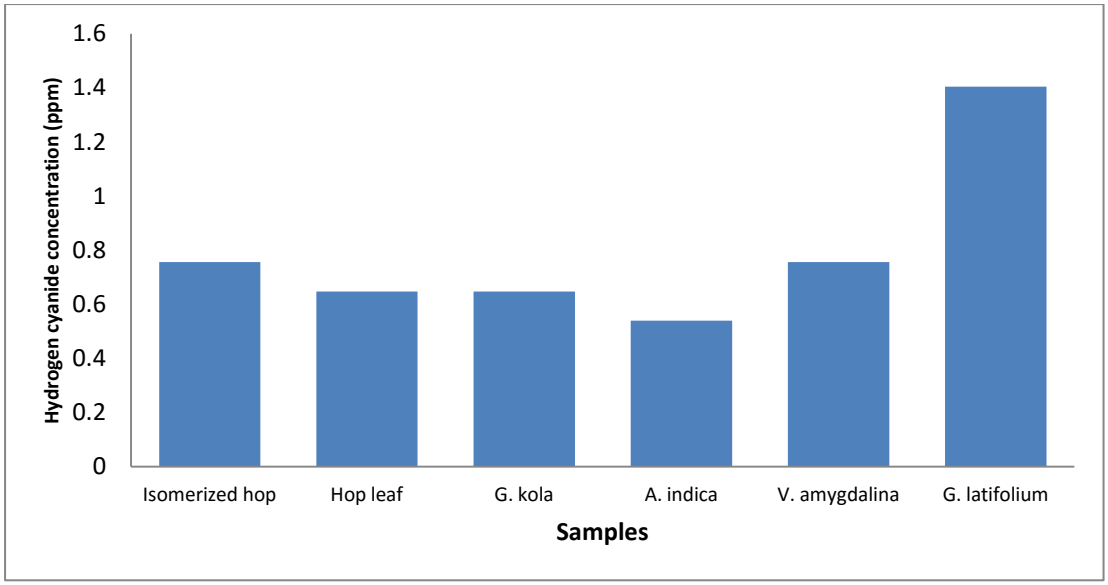


Fig. 4.11: Concentration of Hydrogen cyanide in the samples.

Hydrogen cyanide, sometimes called prussic acid is an inorganic compound. The chemical formula and chemical structure of Hydrogen cyanide are HCN and H – C ≡ N respectively. It is a colourless and extremely poisonous liquid that boils slightly above room temperature at 25.6^{o}C (http://www.wolfranalph.com/ %20input? =boiling point of +Hydrogen + cyanide). A hydrogen cyanide concentration of 3,500 ppm will kill a human in about 60 seconds (Vetter, 2000). 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 (Patnaik, 2002; Gail *et al*., 2007).

4.4 METAL CONTENT OF THE EXTRACTS

4.4.1 Calcium (Ca)

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. It is evident from Table 4.18 that this metal is available in all the samples.

Fig. 4.12 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.

Table 4.18: Concentration of Calcium in the samples

Sample	**Concentration (ppm)**			
	Mean	**St Dev**	**Range**	
Isomerized hop	16.300	4.19	7.70	p_1=0.935>0.05
Hop leaf	17.800	1.73	3.40	p_2=0.746>0.05
G.kola	33.145	4.87	9.54	
A. indica	33.145	2.51	4.84	
V. amygdalina	33.717	3.75	7.04	
G. latifolium	18.400	2.38	4.74	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

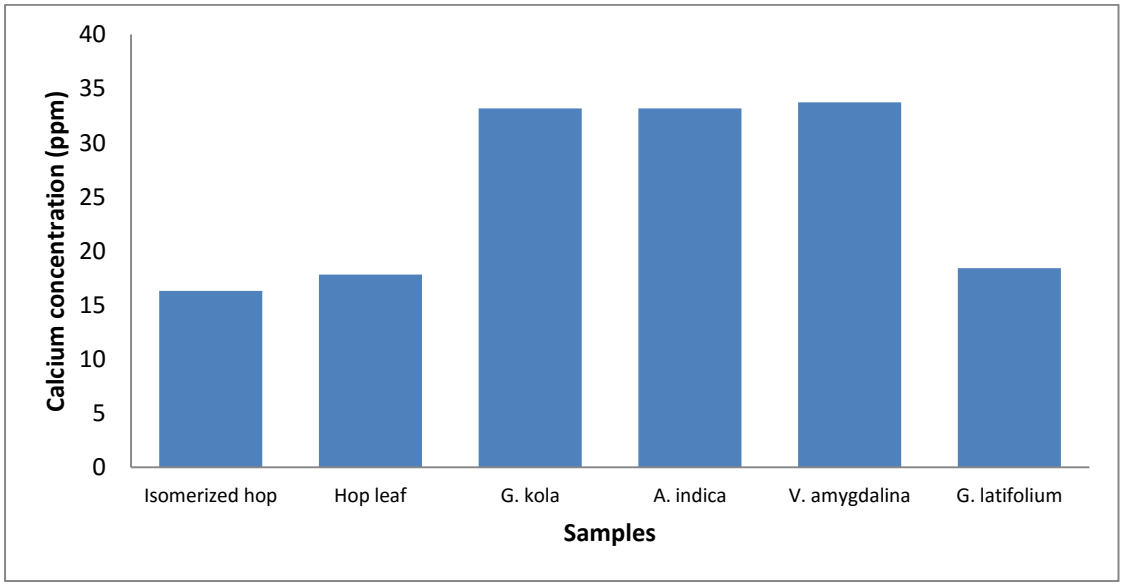


Fig. 4.12: Concentration of calcium in the samples.

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).

4.4.2 Sodium (Na)

The concentration of sodium was virtually the same range for all the samples (Table 4.19) especially isomerized hop, *G. latifolium, A. indica, V. amygdalina* and *G. kola* as can be seen in Fig. 4.13. Sodium plays a major role in controlling blood pressure and blood volume, for proper functioning of muscles and nerves.

Table 4.19: Concentration of Sodium in the samples

Sample	Concentration (ppm)			
	Mean	St Dev	Range	
Isomerized hop	98.245	3.75	7.21	p_1=0.935>0.05
Hop leaf	92.019	7.04	12.24	p_2=0.746>0.05
G.kola	100.151	3.88	6.920	
A. indica	95.122	10.64	19.63	
V. amygdalina	101.263	18.20	36.40	
G. latifolium	95.882	3.360	6.58	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

It is interesting to know 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.

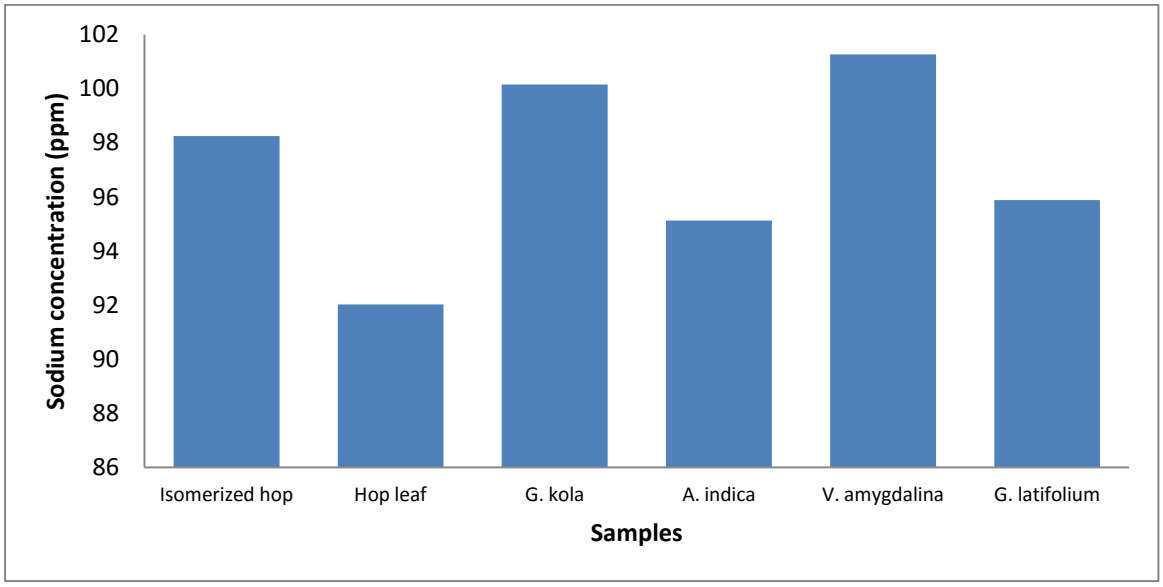


Fig. 4.13: Concentration of Sodium in the samples.

4.4.3 Potassium (K)

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 4.20. 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).

Table 4.20: Concentration of Potassium in the samples

Sample	Concentration (ppm)			
	Mean	**St Dev**	**Range**	
Isomerized hop	206.838	12.050	21.650	p_1=0.935>0.05
Hop leaf	8.297	1.276	2.551	p_2=0.746>0.05
G.kola	82.737	7.140	14.150	
A. indica	206.838	5.200	9.940	
V. amygdalina	60.240	8.670	15.020	
G. latifolium	206.838	9.660	18.560	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

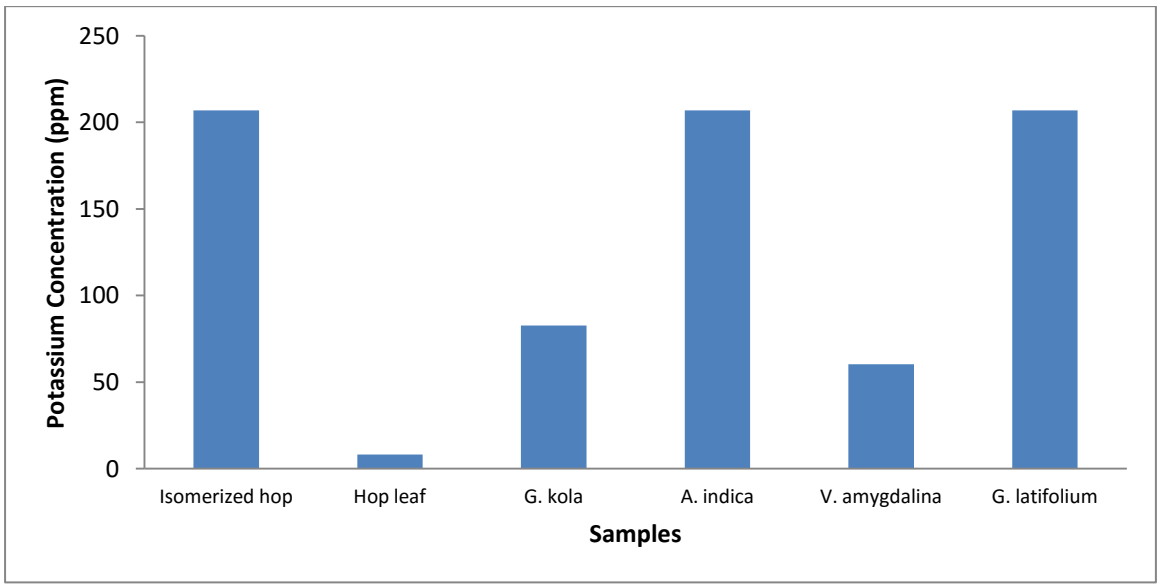


Fig. 4.14: Concentration of Potassium in the samples.

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). Hence, Fig. 4.14 shows that *G. latifolium* and *A. indica* could substitute isomerized hop.

4.4.4 Magnesium (Mg)

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.

Table 4.21: Concentration of Magnesium in the samples

Sample	Concentration (ppm)			
	Mean	St Dev	Range	
Isomerized hop	19.331	3.840	7.530	p_1=0.935>0.05
Hop leaf	21.113	2.600	5.070	p_2=0.746>0.05
G.kola	21.586	1.329	2.322	
A. indica	20.971	1.034	2.053	
V. amygdalina	20.240	3.490	6.930	
G. latifolium	22.188	3.270	6.350	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

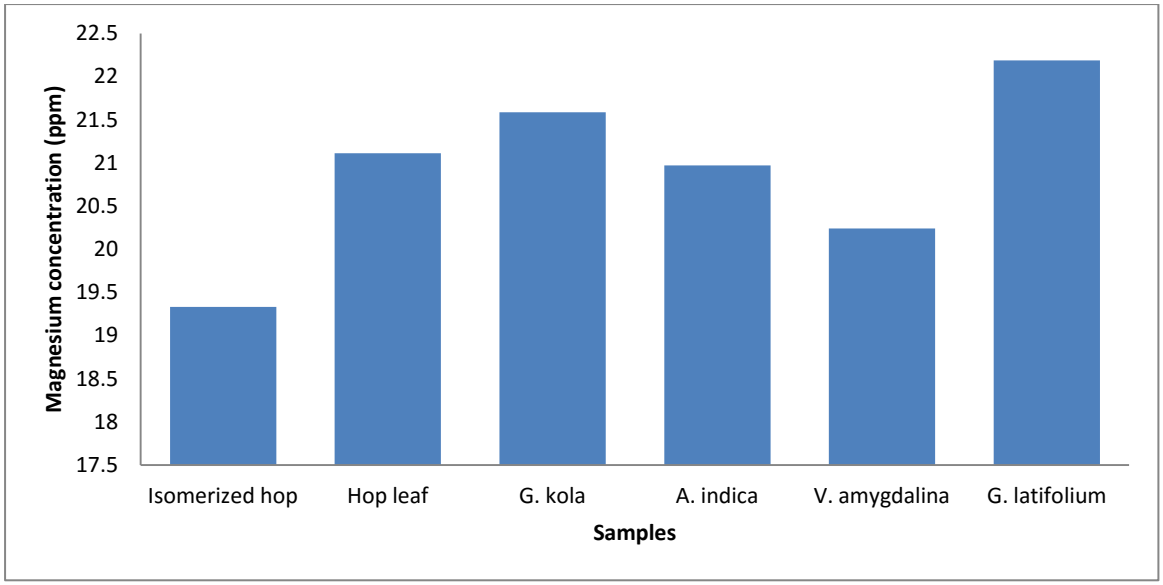


Fig. 4.15: Concentration of Magnesium in the samples.

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 Table 4.21 and Fig. 4.15, magnesium occurred comparably in all the samples. Thus, each can substitute the other in beer production.

4.4.5 Lead (Pb)

This is a highly toxic metal. Lead 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). From this work, lead is

absent in all the samples as expected, Table 4.22 and Fig. 4.16. Thus, each can substitute the other.

Table 4.22: Concentration of Lead in the samples

Sample	Concentration (ppm)			
	Mean	St Dev	Range	
Isomerized hop	0.00	0.00	0.00	p_1=0.935>0.05
Hop leaf	0.00	0.00	0.00	p_2=0.746>0.05
G.kola	0.00	0.00	0.00	
A. indica	0.00	0.00	0.00	
V. amygdalina	0.00	0.00	0.00	
G. latifolium	0.00	0.00	0.00	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

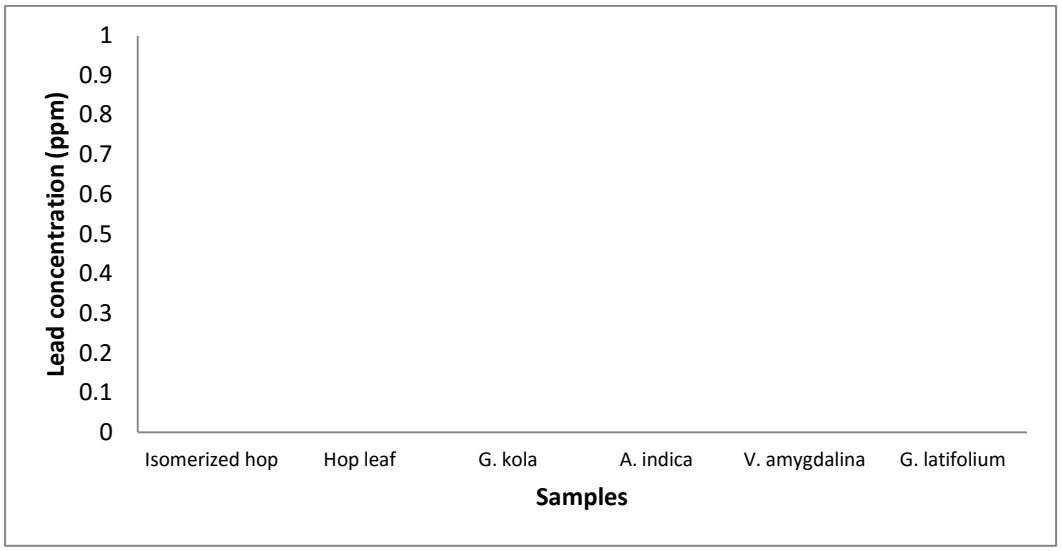


Fig. 4.16: Concentration of lead in the samples.

4.4.6 Manganese (Mn)

This metal was virtually absent in all the samples, Table 4.23 and Fig. 4.17, except in *G. latifolium* where it occurred with some prominence. Thus, the other samples could substitute one another.

Table 4.23: Concentration of Manganese in the samples.

Sample	Concentration (ppm)			
	Mean	St Dev	Range	
Isomerized hop	0.426	0.0295	0.058	p_1=0.935>0.05
Hop leaf	0.850	0.1521	0.275	p_2=0.746>0.05
G.kola	1.038	0.1601	0.301	
A. indica	0.667	0.0800	0.153	
V. amygdalina	0.782	0.1284	0.237	
G. latifolium	38.628	5.7500	11.060	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

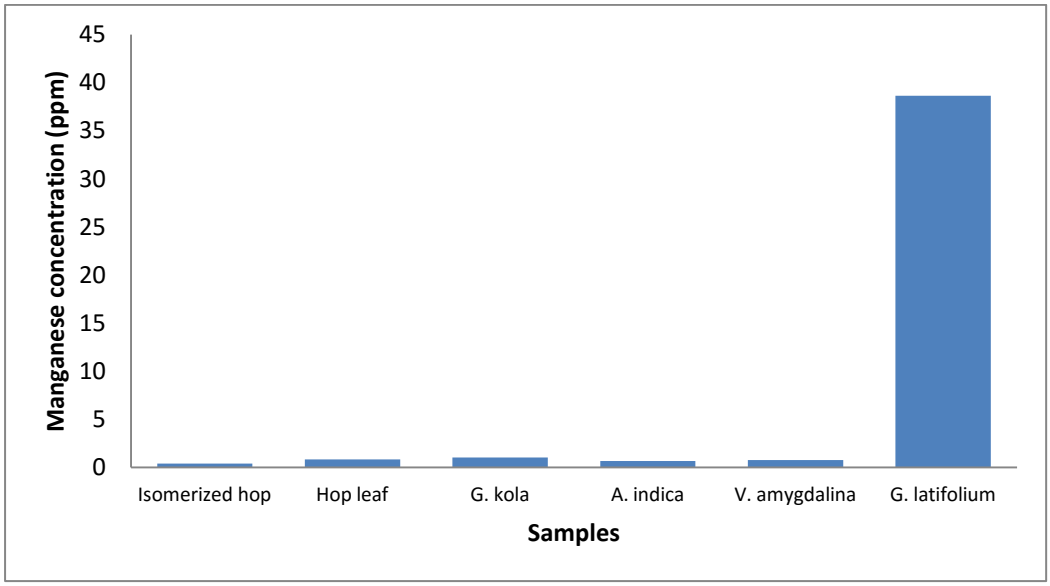


Fig. 4.17: Concentration of manganese in the samples.

Manganese is a mineral element that is both nutritionally essential and potentially toxic. Manganese (Mn) 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).

4.4.7 Cobalt (Co)

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*, Table 4.24 and Fig. 4.18. 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.

Table 4.24: Concentration of Cobalt in the samples

Sample	Concentration (ppm)			
	Mean	**St Dev**	**Range**	
Isomerized hop	0.012	0.00265	0.005	p_1=0.935>0.05
Hop leaf	0.008	0.00265	0.005	p_2=0.746>0.05
G.kola	0.002	0.00173	0.003	
A. indica	0.019	0.00755	0.015	
V. amygdalina	0.000	0.00000	0.000	
G. latifolium	0.004	0.00173	0.003	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

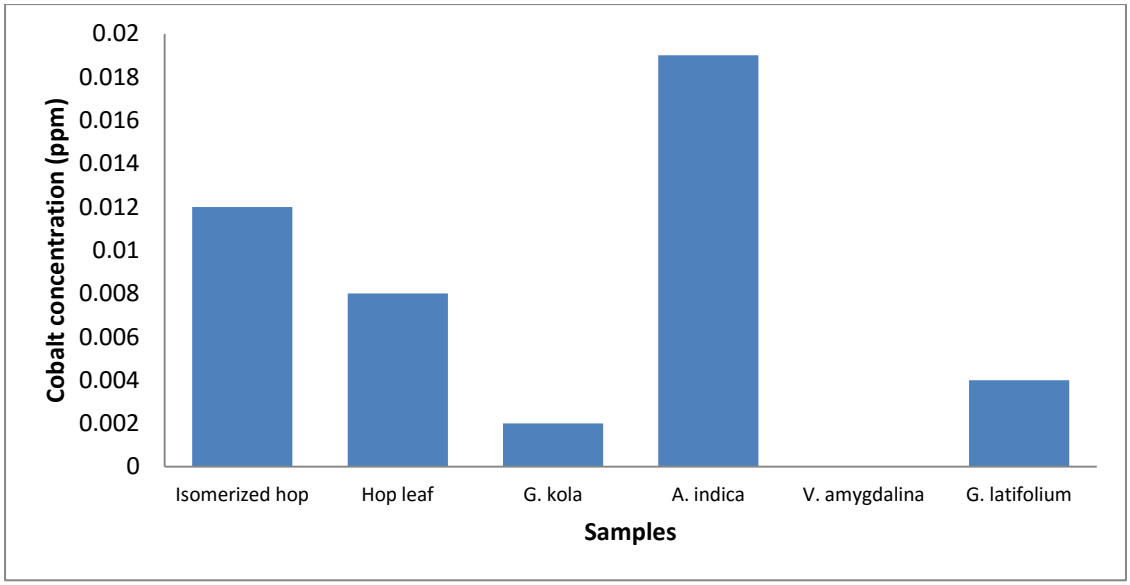


Fig. 4.18: Concentration of cobalt in the samples.

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).

4.4.8 Zinc (Zn)

The concentrations of this metal in hop leaf and *A. indica* were comparatively uniform (Table 4.25 and Fig. 4.19). 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.

Table 4.25: Concentration of Zinc in the samples

Sample	Concentration (ppm)			
	Mean	**St Dev**	**Range**	
Isomerized hop	0.963	0.0730	0.132	p_1=0.935>0.05
Hop leaf	1.985	0.1540	0.307	p_2=0.746>0.05
G.kola	6.072	1.7120	3.192	
A. indica	1.611	0.0930	0.185	
V. amygdalina	1.090	0.0828	0.163	
G. latifolium	17.944	1.6790	2.918	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

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).

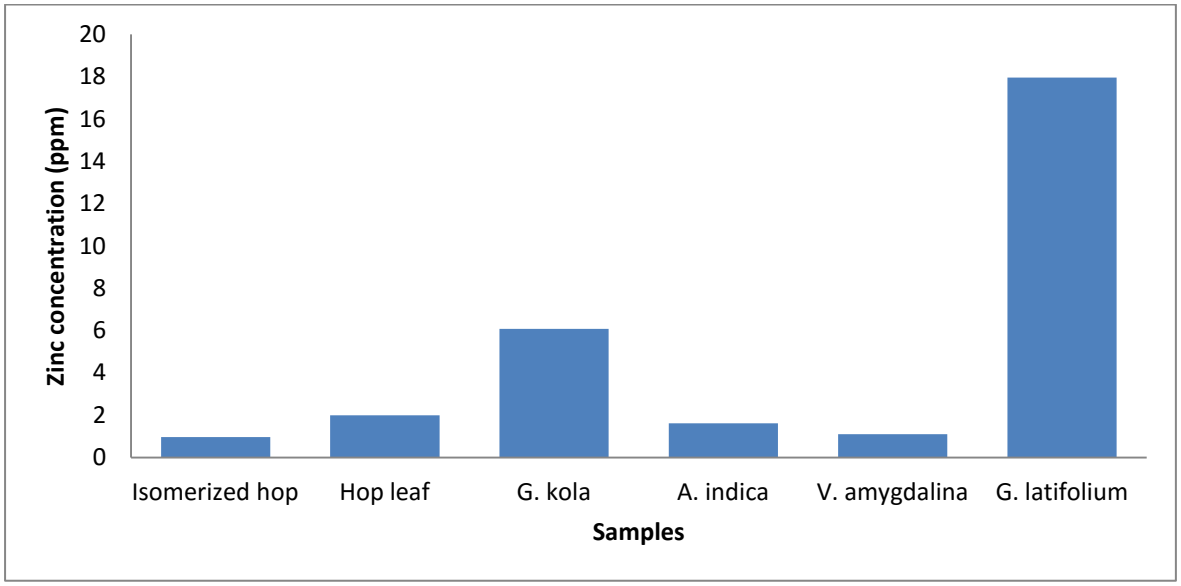


Fig. 4.19: Concentration of zinc in the samples.

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).

4.4.9 Mercury (Hg)

As expected, mercury was virtually absent in most of the samples, Table 4.26 and Fig. 4.20, 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.

Table 4.26: Concentration of Mercury in the samples

Sample	**Concentration (ppm)**			
	Mean	**St Dev**	**Range**	
Isomerized hop	0.000	0.000	0.000	p_1=0.935>0.05
Hop leaf	0.000	0.000	0.000	p_2=0.746>0.05
G.kola	0.000	0.000	0.000	
A. indica	1.127	0.3720	0.681	
V. amygdalina	0.390	0.0361	0.070	
G. latifolium	0.000	0.000	0.000	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

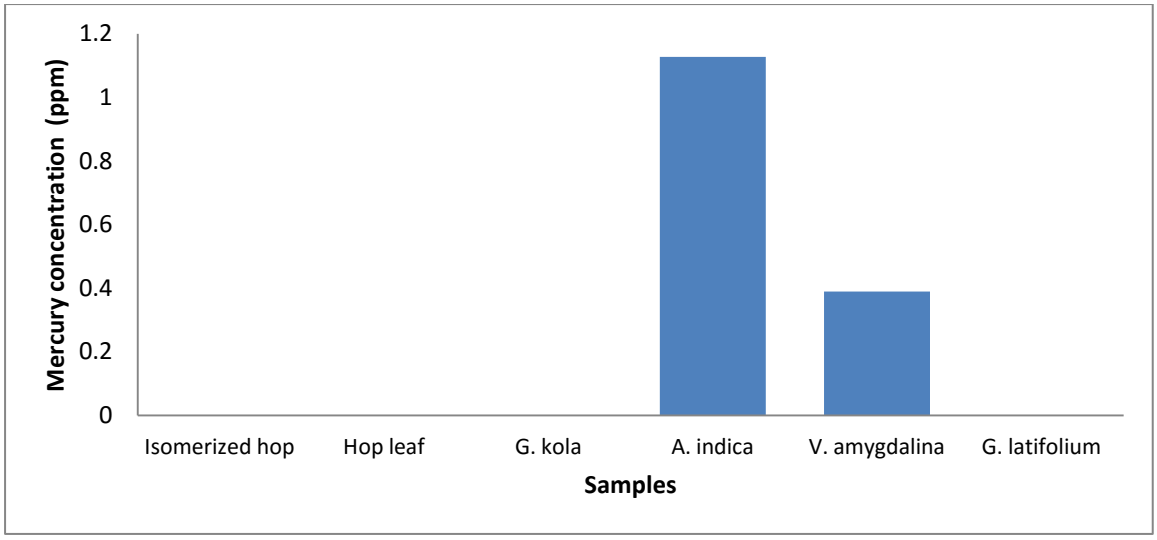


Fig. 4.20: Concentration of mercury in the samples.

4.4.10 Iron (Fe)

Iron was low in all except in *G. latifolium*, Table 4.27 and Fig. 4.21. 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.

Table 4.27: Concentration of Iron in the samples

Sample	Concentration (ppm)			
	Mean	St Dev	Range	
Isomerized hop	0.547	0.08060	0.150	p_1=0.935>0.05
Hop leaf	0.815	0.17900	0.330	p_2=0.746>0.05
G.kola	1.620	0.8540	0.170	
A. indica	2.526	0.11490	0.225	
V. amygdalina	0.159	0.00656	0.013	
G. latifolium	8.614	0.60900	1.217	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

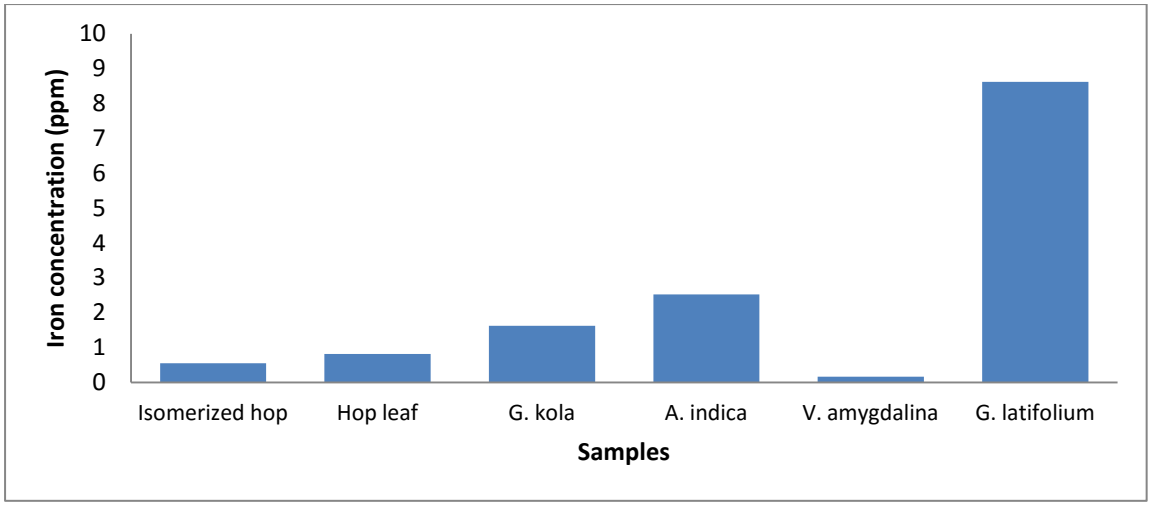


Fig. 4.21: Concentration of iron in the samples.

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). 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.

4.5 BREWING OF BEER

4.5.1 Malting Process

The result of malting show a decrease in weight of the grains from 300g to 281.19g, Table 4.28, after five days of germination representing a malting loss of 6.27%.

Table 4.28: Malting Loss for CSR01 Sorghum Variety

Weight of Grain after Sorting (g)	Weight of Grain after 5 days of Germination (g)	Malting Loss (%)
300	281.19	6.27±0.524

This result is in agreement with that obtained by Archibong *et al*., (2009) in their malting properties of SSV 200504 sorghum variety. Those authors had reported a malting loss of 5.60% after 5 days of germination (Table 4.29).

Table 4.29: Malting loss for SSV 200504 Sorghum Variety

Days of Germination	Malting Loss (%)
1	3.80
2	4.20
3	4.62
4	4.92
5	5.60
6	5.88

Source: Archibong *et al*., 2009.

4.5.2 Mashing Programme

Table 4.30 shows that the time taken by the mash to get from 40^{o}C to 55^{o}C; 55^{o}C to 65^{o}C; 65^{o}C to 72^{o}C; 72^{o}C to 60^{o}C and 60^{o}C to 75^{o}C is 12 minutes, 10 minutes, 15 minutes, 25 minutes and 10 minutes respectively. Fig. 4.22 shows the mashing regime for wort production.

Table 4.30: Temperature regime, time taken to attain the temperature and rest time at attained temperature

Temp. Regime (^{o}C)	Time taken (mins)	Rest time (mins)
25-40	-	30
40-55	12	30
55-65	10	30
65-72	15	10
72-60	25	30
60-75	10	10

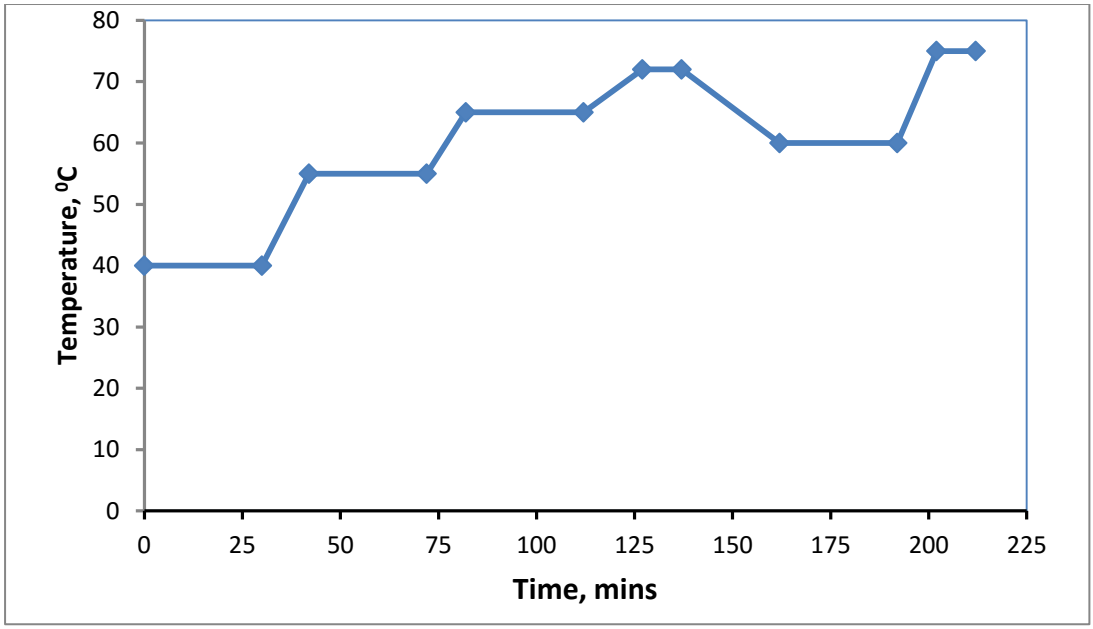


Fig. 4.22: Mashing regime for wort production.

The graph above shows that mashing of the milled malt starts at 40^{o}C and rested for 30 minutes at this temperature. It took 12 minutes (30 -42) before it attained a temperature regime of 55^{o}C and rested for 30 minutes (42-72). It took the mash another 10 minutes (72-82) to attain the temperature of 65^{o}C where it was allowed to rest for 30 minutes (82 – 112) before it was raised to 72^{o}C after an interval of 15 minutes (112-127). The mash rested at 72^{o}C for 10minutes (127-137) before the temperature was reduced to 60^{o}C. The time taken to attain this

temperature is 25 minutes (137-162) and allowed to rest for 30 minutes (162-192). It took another 10 minutes (192-202) to attain a higher temperature of 75^{o}C where it was allowed to rest for 10 minutes (202-212). The results in Fig. 4.22 are consistent with that of Archibong and Onuorah (2010), Fig. 4.23 indicating that there is no significant difference in the mashing regimes.

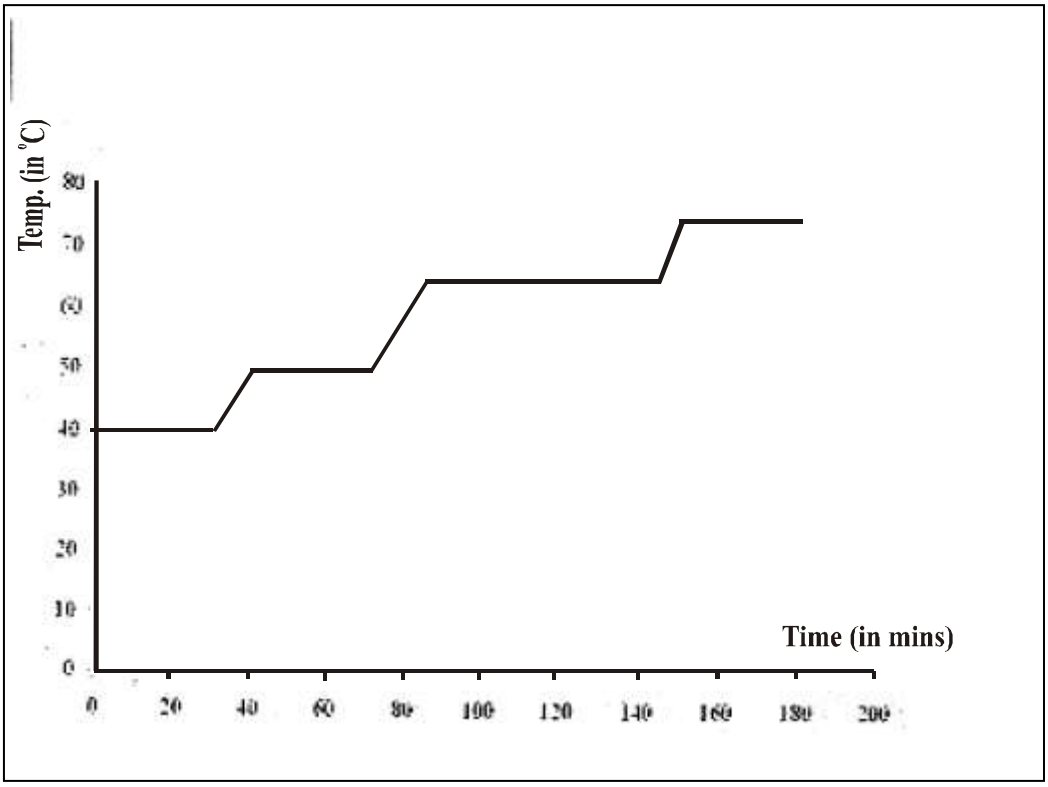


Fig. 4.23: Mashing Regime for SSV 200504 Sorghum variety.
Source: Archibong and Onuorah, (2010).

4.6 PHYSICAL AND CHEMICAL PROPERTIES OF THE BEER SAMPLES

4.6.1 Alcohol Content

Table 4.31 and Fig. 4.24 show that percentage alcohol was virtually in the same range in all the samples especially samples A and D with alcohol content of 3.75 %v/v. All the other samples contained equal alcohol content of 3.43 %v/v.

This result is in agreement with the report of Hough *et al*., (1982) in their investigation of alcohol content in British beers.

Table 4.31: Alcohol content of the beer samples

Beer Sample	**Alcohol (% v/v)**			
	Mean	**St Dev**	**Range**	
A= Isomerized hop	3.75	0.00	0.00	p_1=0.705>0.05
B= Hop leaf	3.43	0.00	0.00	p_2=0.743>0.05
C= *G. kola*	3.43	0.00	0.00	* =5% v/v
D= *A. indica*	3.75	0.00	0.00	
E= *V. amygdalina*	3.43	0.00	0.00	
F= *G. latifolium*	3.43	0.00	0.00	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants
* = NAFDAC's maximum allowed alcohol content in Nigerian beers

The authors had reported that the majority of beers tested contained between 2.5 %v/v and 5 %v/v alcohol. However none of the alcohol content of all the beer samples is up to Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC) maximum allowed alcohol content of 5 %v/v.

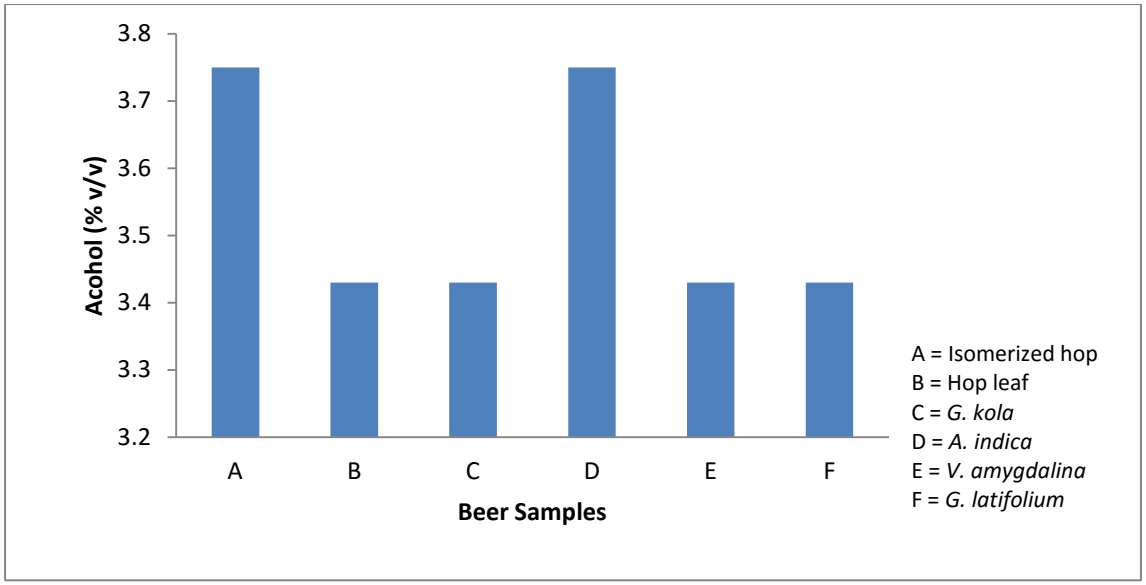


Fig. 4.24: Alcohol content of the beer samples.

The alcohol content of beer is usually regarded as the measure of its strength and therefore, the more the alcohol content, the stronger the beer and vice versa. The comparability of the results indicates that fermentation of the wort was done under the same condition and that hops do not affect the alcohol content of beers.

4.6.2 Total acidity

The total acidity in all the beer samples varied between 0.324% and 0.132 % with the highest percentage of total acidity in sample D being 0.324% and lowest value of 0.132% in sample C. Table 4.32 shows that percentage total acidity in samples A and B are respectively 0.228% and 0.144%, whereas that in samples E and F contain equal content of total acidity of 0.288% each.

Table 4.32: Total acidity of the beer samples

Beer Sample	Total acidity (% v/v)			
	Mean	St Dev	Range	
A= Isomerized hop	0.228	0.06490	0.126	p_1=0.705>0.05
B= Hop leaf	0.114	0.02350	0.047	p_2=0.743>0.05
C= *G. kola*	0.132	0.01442	0.028	* =5%
D= *A. indica*	0.324	0.03260	0.065	
E= *V. amygdalina*	0.288	0.06290	0.124	
F= *G. latifolium*	0.288	0.05070	0.094	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants
* = NAFDAC's minimum allowed total acidity in Nigerian beers

From Fig. 4.25, it is evident that the percentage total acidity of all the samples are comparably within the same range. Interestingly, all the beer samples exceeded the 0.1% minimum allowed total acidity of NAFDAC's recommendation in lager beers in Nigeria (NAFDAC, 2002).

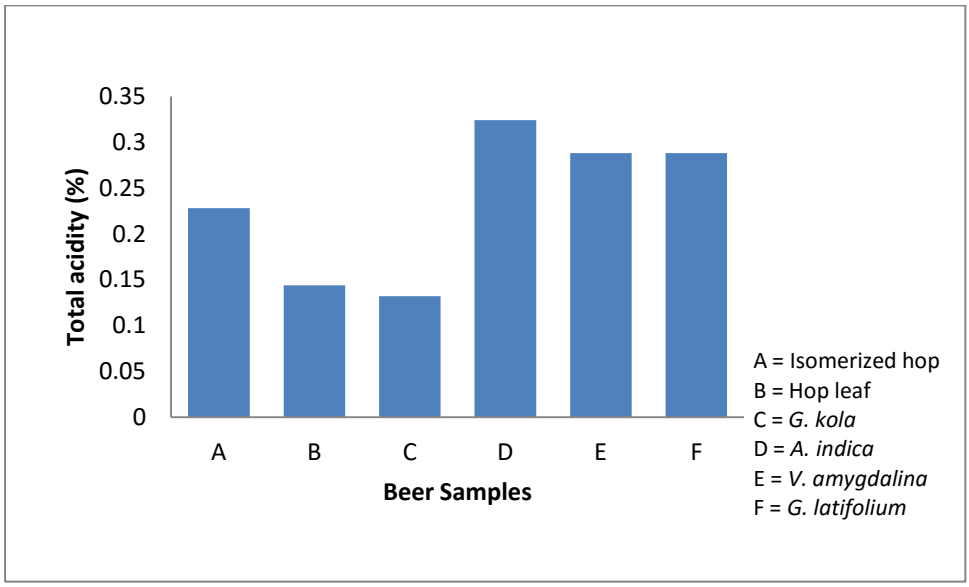


Fig. 4.25: Total acidity in the beer samples

4.6.3 pH

Table 4.33 and Fig. 4.26 show that the pH of the beer samples are comparably the same. Sample B has the highest pH of 5.68 (less acidic). Sample A and C have the same pH value of 5.57. Sample D has the lowest pH value of 5.47 (more acidic) while samples E and F have pH values of 5.50 and 5.49 respectively. This is an indication that all the samples could substitute one another in beer brewing. Interestingly, the pH values are within the NAFDAC standard for lager beers. Worts with pH (5.0 – 5.5) have better protein precipitation and break formation (Kraus-Weverman, 1998).

Table 4.33: pH of the Beer Samples

Beer Sample	**pH at 24^{o}C**			
	Mean	**St Dev**	**Range**	
A= Isomerized hop	5.57	1.149	2.18	p_1=0.705>0.05
B= Hop leaf	5.68	0.522	0.99	p_2=0.743>0.05
C= *G. kola*	5.57	0.960	1.92	
D= *A. indica*	5.47	0.451	0.90	
E= *V. amygdalina*	5.50	0.756	1.37	
F= *G. latifolium*	5.49	1.208	2.19	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

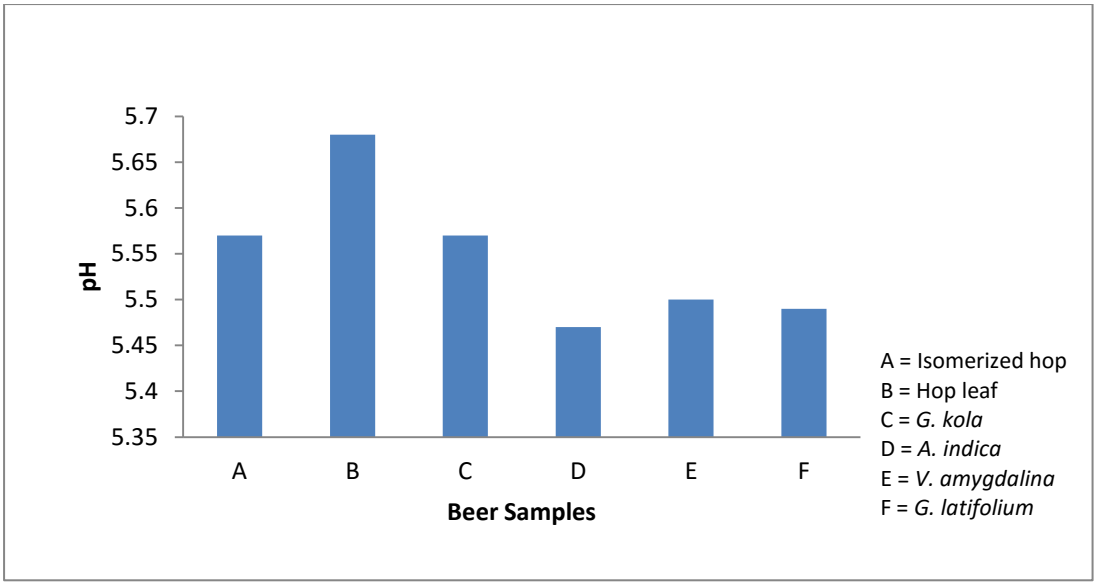


Fig. 4.26: pH of the beer samples

4.6.4 Turbidity

The turbidity of all the samples as presented in Table 4.34 ranged between 125 and 5 NTU. Sample E was virtually the clearest and D, the most turbid (cloudy). Samples B, C, and D were 100, 110, and 125 respectively, all in Nephelometric Turbidity Unit (NTU) and were especially high compared with the turbidity in samples E (5 NTU) and F (6 NTU). The turbidity in sample A was 50 NTU. These results are not in agreement with the turbidity standards (0.15 NTU) for drinking water in the United State (EPA, 2009).

Table 4.34: Turbidity of the beer samples

Beer Sample	Turbidity (NTU)			
	Mean	St Dev	Range	
A= Isomerized hop	50	25.000	50.0	p_1=0.705>0.05
B= Hop leaf	100	25.000	50.0	p_2=0.743>0.05
C= *G. kola*	110	10.000	20.0	
D= *A. indica*	125	5.000	10.0	
E= *V. amygdalina*	5	2.500	5.00	
F= *G. latifolium*	6	1.323	2.5	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

Fig. 4.27 shows that the turbidity in samples B, C and D are comparable to one another; samples E and F are also comparable to each other while sample A is not comparable to any of the samples.

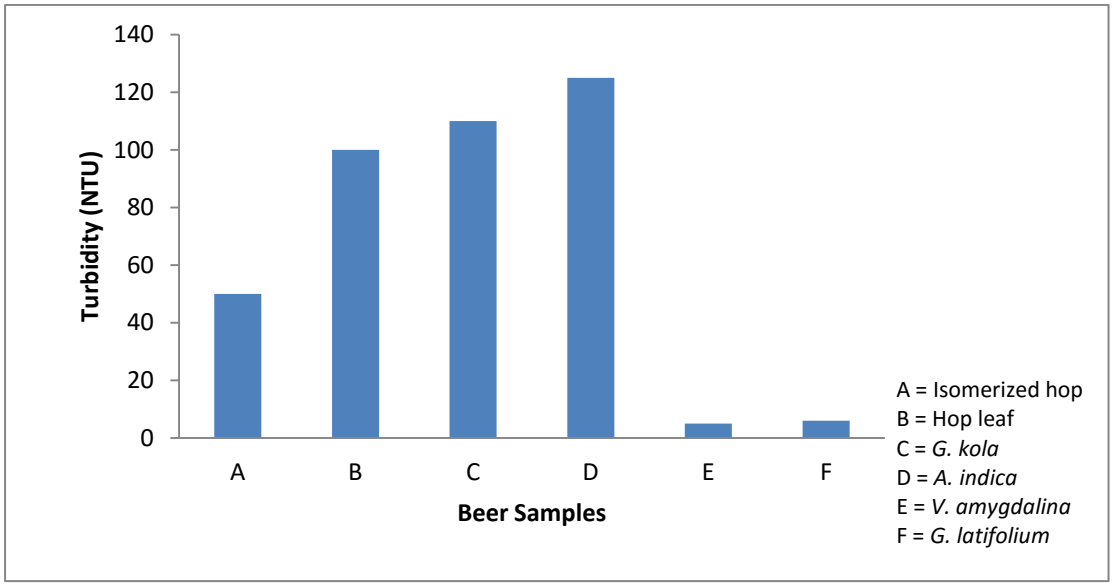


Fig. 4.27: Turbidity of the beer samples

These discrepancies that exist in turbidity values of the beer samples could easily be explained by the fact that length of time each beer sample was exposed to the atmosphere during hopping was not constant. During each period, fugitive harmful organisms such as bacteria, viruses, protozoa, moulds, and wild yeasts could infect the beer. The more the beer is exposed to the atmosphere, the more the loads of these organisms and of course the more the beer develops a biological haze and goes turbid.

These results could explain the reason why excess consumers of beer often complain about gastrointestinal diseases because in drinking water, the higher the turbidity level, the higher the risk that people may develop gastrointestinal diseases (Mann *et al*., 2007).

4.6.5 Total Solids

From Table 4.35, the percentage total solids ranged between 3.86 and 8.16 with sample D having the highest percentage of 8.16 and the lowest, sample B, with a value of 3.86%.

Table 4.35: Total Solids in the beer samples

Beer Sample	Total solids (%)			
	Mean	St Dev	Range	
A= Isomerized hop	6.34	0.2950	0.52	p_1=0.705>0.05
B= Hop leaf	3.56	0.0529	0.10	p_2=0.743>0.05
C= *G. kola*	3.86	0.0964	0.18	* =5%
D= *A. indica*	8.16	0.1442	0.28	
E= *V. amygdalina*	4.72	0.0200	0.04	
F= *G. latifolium*	5.85	0.0500	0.10	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants
*=NAFDAC's permissible maximum unit

The percentage total solids of the beer in samples B, C, and E are within the permissible maximum limits of total solids in beer. The National Agency for Food and Drug Administration and Control (NAFDAC)'s permissible maximum unit of total solids in beer is 5%. The percentage total solids in samples A and D are above the permissible limit while that in F is slightly above the limit.

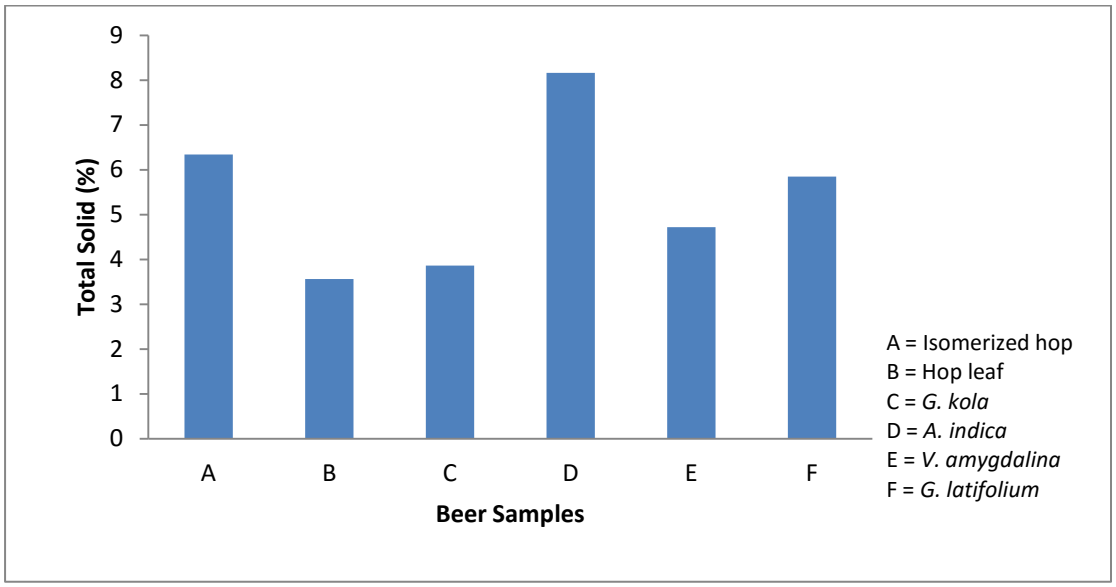


Fig. 4.28: Total solids in the beer samples

Fig. 4.28 shows that the total solids in sample D were more than twice those in samples B and C. This observation reveals that hops contribute to percentage total solids in beer. Total solids in A, E, and F are virtually within the same range. However, the results are in agreement with the report of O'Rourke (1998) on water content of beer. O'Rourke has reported that beers contain more than 90% water.

4.6.6 Micro metals (Arsenic, Cadmium and Copper)

The results from Table 4.36 and Fig. 4.29 show that the concentrations of these elements in the finished beer samples differ among themselves because metals in beer are derived from various raw materials, equipment and brewing processes (World Health Organization (WHO), 2001).

Table 4.36: **Micro metals in the Beer Samples**

	Metals (ppm)			
Beer Sample	As	Cd	Cu	
A= Isomerized hop	1.50±0.265	0.00±0.000	2.70±0.917	p_1=0.705>0.05
B= Hop leaf	1.44±0.1682	0.81±0.0964	1.99±0.185	p_2=0.743>0.05
C= *G. kola*	1.77±0.556	0.97±0.889	2.39±0.1054	
D= *A. indica*	1.52±0.0265	0.96±0.0529	0.10±0.0436	
E= *V. amygdalina*	1.62±0.0721	0.00±0.000	1.32±0.1058	
F= *G. latifolium*	1.60±0.278	0.68±0.052	1.46±0.0693	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

Arsenic (As)

From Table 4.36, arsenic concentration in the samples ranged between 1.44 – 1.77mg/L, with sample C having the highest concentration of 1.77mg/L, and sample B, the lowest concentration of 1.44mg/L. The FAO/WHO maximum permissible limit of arsenic in drinking water is 10µg/L (FAO/WHO, 2011). The arsenic content of the beer samples was above the maximum permissible limit of arsenic in drinking water. In Britain, the level of arsenic in lagers may not exceed 0.2mg/kg (Hough *et al*, 1982). Again, the concentration of arsenic in the beer samples investigated is much more above this level. The explanation for this may be the region of growth of the raw materials used in the production.

Cadmium (Cd)

From the results in Table 4.36 and Fig. 4.29, cadmium concentration in the beer samples ranged between 0.97ppm and not detected with sample C having the highest concentration of 0.97 mg/L and not detected in samples A and E. The concentrations of cadmium in samples B, D and F are 0.81ppm, 0.97ppm and 0.68ppm respectively. These results differ significantly with the result of

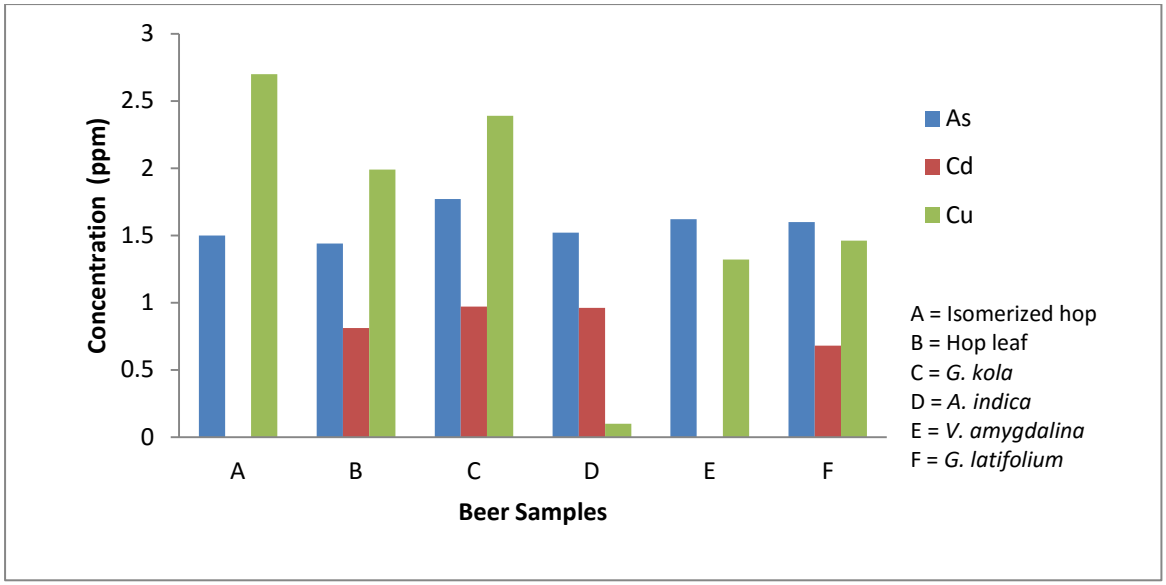


Fig 4.29: Micro metals in the beer samples.

Ubuoh (2013) except in samples A and E. (World Health Organization (WHO) (2001) reported a cadmium content varying from 12.90 – 14.30µg/l in Brazilian beers. Also, the Standard Organization of Nigeria, SON (2003) gave the limit for Cd content in drinking water as Iµg/kg bw/day. All the beer samples examined had Cd concentrations above that in Brazilian beers and the permissible limit in drinking water with the exception of beer samples A and E where Cd was not detected.

Copper (Cu)

Copper content of the beer samples as shown in Table 4.36 varied between 2.70ppm and 0.10ppm, with sample A having the highest concentration of 2.70ppm, and the lowest being sample D with 0.10ppm. The permissible limit for copper in drinking water in Nigeria is 1.0ppm (Standard Organization of Nigeria (SON), 2003; World Health Organization (WHO), 1993) and in Britain, the Food

Standard Committee has recommended limits of 7.0ppm and 5.0ppm for copper and zinc respectively in wines and beers (Hough *et al*, 1982). The Copper content of the beer samples analyzed was above the permissible limit for drinking water in Nigeria except in Sample D but below the limit in British beers.

4.6.7 Bitterness Level

The bitterness level in all the samples ranged between 25.38IBU and 39.62IBU with sample A having the highest bitterness level of 39.62IBU and sample C, the lowest bitterness level of 25.38IBU. Samples B, D, E and F have bitterness levels of 30.91IBU, 33.87IBU, 29.12IBU and 27.56IBU respectively. Table 4.37 and Fig. 4.30 show that bitterness level in all the beer products are virtually in the same range and especially high in sample A.

Table 4.37: Bitterness level of the beer samples

Beer Sample	**Bitterness level (IBU)**			
	Mean	**St Dev**	**Range**	
A= Isomerized hop	39.62	0.541	1.00	p_1=0.705>0.05
B= Hop leaf	30.91	1.012	2.00	p_2=0.743>0.05
C= *G. kola*	25.38	0.541	1.00	
D= *A. indica*	33.87	0.778	1.50	
E= *V. amygdalina*	29.12	0.769	1.42	
F= *G. latifolium*	27.56	2.150	4.30	

P_1=p-value for isomerized hop and the Nigerian plants
P_2=p-value for hop leaf and the Nigerian plants

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

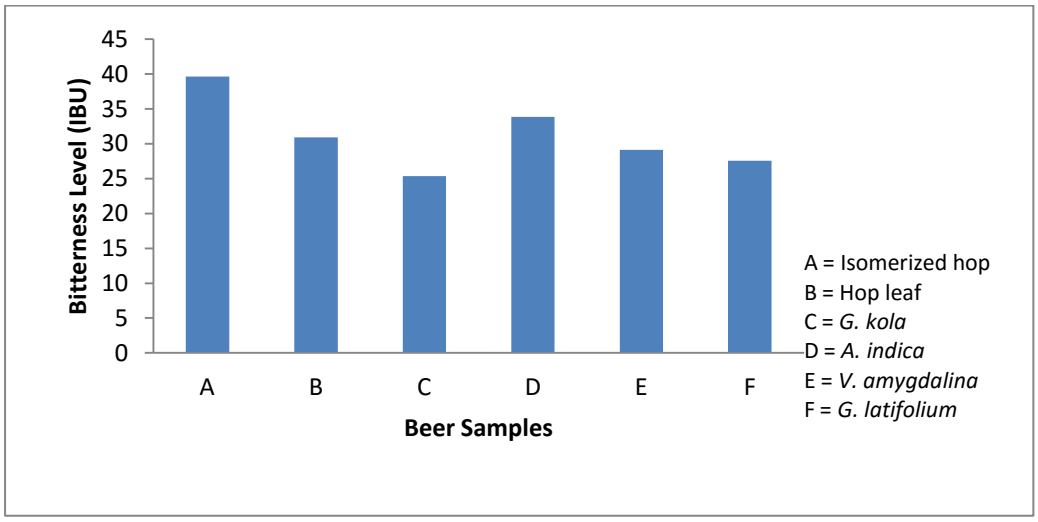


Fig. 4.30: Bitterness levels of the beer samples

4.7: STATISTICAL RESULTS

4.7.1: Ranking of phytochemicals.

Isomerized hop

In the comparison of mean values, *G. kola* has the closest mean value of 2.1175 to isomerized hop's 2.9755 (Appendix 5).The p-value of test is 0.633 (Table 4.38) 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 4.38 ANOVA for comparison of phytochemicals in Isomerized hop and the Nigerian plants

	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 shows 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 (Appendix 5) which shows that the samples are not significantly different from isomerized hop.

Hop leaf

The p-value of the test is 0.645 which is greater than 0.05 (Table 4.39) and then, we have enough evidence to conclude that there is insignificant difference among the samples (hop leaf, *G. kola*, *A. indica, V. amygdalina* and *G. latifolium*).

Table 4.39 ANOVA for comparison of phytochemicals in Hop leaf and the Nigerian plants

	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	655.308	49			

The multiple comparisons using Post Hoc Test (Least Significant Difference) shows that *A. indica* has the highest significance value of 0.637 (Appendix 5) which implies that this plant is closest among all to hop leaf (control). Other substitutes, 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.

4.7.2: Ranking of metals

Isomerized hop

The p-value of the test is 0.935 (Table 4.40) which is greater than 0.05. We then have enough evidence to accept the null hypothesis and conclude that there is insignificant difference among the samples studied.

Table 4.40 ANOVA for comparison of metals in Isomerized hop and the Nigerian plants

	Sum of squares	df	Mean square	F	Sig.
Between Groups	2586.212	4	646.553	0.704	0.935
Within Groups	142709.648	45	3171.326		
Total	145295.860	49			

The output of the Post Hoc Test (Appendix 5) shows that *A. indica* has the highest significance value of 0.939 which implies that the sample is the closest among all the plants to isomerized hop. Others (*G. kola, V. amygdalina* and *G. latifolium*) have significance values less than 0.939 but higher than 0.05. This shows that the samples are not significantly different from isomerized hop.

Hop leaf

The p-value of the test is 0.746 (Table 4.41) which is greater than 0.05. We therefore have enough evidence to accept the null hypothesis and conclude that there is insignificant difference among the plants considered.

Table 4.41 ANOVA for comparison of metals in Hop leaf and the Nigerian plants

	Sum of squares	df	Mean square	F	Sig.
Between Groups	4693.103	4	1173.276	0.486	0.746
Within Groups	108639.142	45	2414.203		
Total	11333.245	49			

It is evident from the Post Hoc Test (Appendix 5) that in the comparison of hop leaf with the Nigerian plants, *V. amygdalina* has the highest significance value of 0.734 which implies that *V. amygdalina* is the closest among all the samples to hop leaf (control). Others (*G. kola, A. indica* and *G. latifolium*) have significant

values less than 0.734 but higher than 0.05. This shows that the plants are not significantly different from hop leaf.

4.7.3 Ranking of physicochemical properties of brewed beers

Isomerized hop

The p-value of the test as shown in Table 4.42 is 0.705 which is greater than 0.05. We then have enough evidence to accept the null hypothesis and conclude that there is no significant difference among the samples studied.

Table 4.42: ANOVA for comparison of physicochemical properties of beers brewed with Isomerized hop and the Nigerian plants

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	1497.217	4	374.304	0.543	0.705
Within Groups	27565.760	40	689.144		
Total	29062.978	44			

The output of the post Hoc Test (Appendix 5) shows that *G. kola* has the highest significance value of 0.696 which implies that the plant is the closest among all the samples to isomerized hop. Other Nigerian plants *(A. indica, V. amygdalina and G. latifolium)* have significance values less than 0.696 but greater than 0.05. This implies that all the plants are insignificantly different from isomerized hop.

Hop leaf

From table 4.43, it is seen that the p-value of the test is 0.734 which is greater than 0.05. We therefore have enough evidence to accept the null hypothesis and conclude that there is no significant difference among the plants considered.

Table 4.43 ANOVA for comparison of physicochemical properties of beers brewed with Hop leaf and the Nigerian plants.

	Sum of squares	df	Mean square	F	Sig.
Between Groups	1632.103	4	408.026	0.490	0.743
Within Groups	33324.703	40	833.118		
Total	34956.806	44			

It is seen from the post Hoc Test (Appendix 5) that in the comparison of hop leaf with the Nigerian plants, *G. kola* has the highest significance value of 0.964 which implies that *G. kola* is the closest among the plants to hop leaf (control). Other Nigerian plants *(A. indica, V. amygalina* and *G. latifolium)* have significant values less than 0.734 which are higher than 0.05. This means that the plants are not significantly different from hop leaf.

4.7.4 Overall ranking

Isomerized hop

The p-value of the test is 0.878 (Table 4.44) which is higher than 0.05. We conclude that there is insignificant difference among the samples.

Table 4.44 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 (Appendix 5) 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 4.45) 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 4.45 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 (Appendix 5). 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*.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The following deductions can be made from this work:

- Chemical constituents present in imported hops (control) and the Nigerian plants differed significantly. Imported hops contained seven constituents that were not found in the Nigerian plants. Four constituents were found to be present in both hop leaves/processed female hop inflorescence and the Nigerian plants. There were also some constituents which were present in the Nigerian plants but absent in imported hops.
- 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.
- The results of the AAS showed that the concentrations of the metals (calcium, sodium, potassium, magnesium, manganese, lead, cobalt, zinc, mercury and iron) occur comparably well in all the samples except lead which was absent in all and mercury which was present in only *A. indica and V. amygdalina,* nonetheless in negligible amounts.
- The physical and chemical parameters [total solids, alcohol content, pH, turbidity, total acidity, inorganic micro metals (arsenic, cadmium and copper)

and bitterness level] investigated in the beers brewed with extracts of Nigerian plants showed no significant difference from those of the controls.

- Ranking revealed that the order of closeness to isomerized hop extract is *G. latifolium* (0.971) > *G. kola* (0.860) > *A. indica* (0.751) > *V. amygdalina* (0.551) while that to hop leaf extract is *V. amygdalina* (0.958) > *G. kola* (0.686) > *G. latifolium* (0.578) > *A. indica* (0.347).

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. 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.

5.2 CONTRIBUTION TO KNOWLEDGE

This study has quantitatively established the chemical constituents of hop leaves/processed female hop inflorescence as contrasted with such constituents in four Nigerian possible hop substitutes. The constituents (hexadecanoic acid, octadecenoic acid methyl ester, octadecanoic acid methyl ester and 6-octadecenoic acid) are contained in all the extracts while 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 and 1,2-benzenedicarboxylic acid, bis(-2-ethyl hexyl) ester are contained in only

imported hops. The relative proportion of the constituents contained in all the extracts was established thus: hexadecanoic acid [isomerized hop (7.84%), hop leaf (9.54%), *G. kola* (9.30%); *A. indica* (9.57%), *V. amygdalina* (9.23%), *G. latifolium* (10.69%)]; octadecenoic acid methyl ester [isomerized hop (3.69%), hop leaf (3.14%), *G. kola* (2.84%), *A. indica* (4.34%), *V. amygdalina* (5.12%), *G. latifolium* (0.77%)]; octadecanoic acid methyl ester [isomerized hop (1.21%), hop leaf (2.45%), *G. kola* (1.62%), *A. indica* (2.36%),*V. amygdalina* (2.63%), *G. latifolium* (0.65%)] and 6-octadecenoic acid [isomerized hop (28.96%), hop leaf (43.55%), *G. kola* (44.09%), *A. indica* (44.96%), V. *amygdalina* (43.42%), *G. latifolium* (44.61%)].

Furthermore, the study has demonstrated statistically that the difference established by using GC/MS, AAS and other phytochemical analyses does not affect the physicochemical quality of brewed beer significantly.

5.3 RECOMMENDATIONS

We recommend the following:

- Further studies to investigate so many other physicochemical properties such as shelf life, taste, odour, mould count, coliform count, *E. coli* count and aerobic mesophilic count is strongly recommended.
- Academic activity in the area of mixtures/blends of plant species which mimic hop taste is also recommended.

REFERENCES

AOAC (1980). Association of Official Analytical Chemists. Official Methods of Food Analysis, 19th Edition, Washington, D.C.

AOAC Method 970.14, Haze (Total) of Beer after Chilling, AOAC Intrenational, www.aoc.org.

Adama, K.K., Oberafo, A.A., and Dika, S.I. (2011). Bitter leaf as local substitute for hops in the Nigerian brewing industry. *Arch. Appl. Res*. 3(4): 388 – 897.

Adesuyi, A.O., Elumm, I.K., Adavamola, F.B. and Nwokocha, A.G.M. (2012). Nutritional and Phytohchemical Screening of *Garcinia kola* Adv. *J. Sci*. *Technol*, 4(1):9-14.

Agba-Egbe, T. and Lape, M.I. (2006). The effects of processing techniques in reducing cyanogens levels during the production of some Cameroonian cassava foods. *Journal of Food Composition and Analysis;* 19:354-363.

Ajebesone, P.E. and Aina, J.O. (2004). Potential African Substitutes for hops in Tropical Beer Brewing. *J. Food Technol*., *in Afr*., 9(1): 13-16.

Akerele, O. (1993). Summary of World Health Organization (WHO), Guidelines for the Assessment of Herbal Medicines. Herbal Gram., 22:13-28.

Akinkpelu, D.A. (1999). Antimicrobial activity of Vernonia amygdalina leaves. Fitoterapia – *The Journal for the study of Medicinal Plants*, 70 (4): 432-437.

Akuodor, G.C., Idris-Usman, M.S., Mba, C.C., Meqwas, U.A., Akpan, J.L., Ugwu, T.C., Okoroafor, D.O. and Osunkwo, U.A. (2010). Studies on anti-ulcer, analgestic and antipyretic properties of the ethanolic leaft extract of *Gongronena latifolium* in rodents. *African Journal of Biotechnology* 9 (15): 2316-2321.

Alderton, Bailey, G.E., Lewis, J.C. and Stitt, F. (1954). Analyt. Chem., *26*, 983.

American Society of Brewing Chemists (1976). *Methods of Analysis*, 7th Edn., American Society of Brewing Chemists, St. Paul, Minesota.

American Society of Brewing Chemists (ASBC) Beer-23A.

Amerine, M.A., Pangborn, R.M. and Roessler, E.B. (1965). *Principles of Sensory Evaluation of Food*, Academic Press, New York.

Andrews, J. (2004). A Review in Progress of Mash Separation Technology, MBAA Technical Quarterly, 41(1).

Archibong, E.J., Odibo, F.J.C. and Awah, N.S.(2009). Effects of germination days on the malting properties of three (3) new sorghum varieties (ICSVIII, SSV200504 and SSV2OO503). *World Journal of Biotechnology.*40(1):1578-1583.

Archibong, E.J. and Onuorah, S.C. (2010). Effects of Mashing regime on wort characteristics of three improved sorghum varieties (ICSV III, SSV200504 and SSV200503). *Journal of Applied Sciences*.13(1). pp. 9311 -9319.

Aregheore, E.M., Makkar, H.P.S. and Becker, K. (1997). Chemical Composition and Tannins in leaves of Some browse plants from Delta Central Nigeria eaten by ruminants. *Proceedings of the Society of Nutritional Physiology*, Frankfurt, Germany, p. 112.

Arnold, J. (2005). Origin and History of Beer and Brewing: From Prehistoric Times to the Beginning of Brewing Science and Technology: Cleveland, Ohio: Thieme Publishers.

Aschner, M. (2010). Vanderbilt University http://www.vitamineher buniversity.com/images/spacer.gff Medical Centre.

Ashurst, P.R. (1971). Hops and Their Use in Brewing. Modern Brewing Technology, edited by W.P.K. Findlay. Cleveland, Ohio: The Macmillan Press.

Ault, R., and Newton, R. (1974). *Modern Brewing Technology*. London, United Kingdom: Findlay Macmillan Press.

Ayoola, G.A., Sofidiya, T., Odukoya, O. and Coker, H.A.B. (2006). Phytochemical Screening and Free Raidcal Scavenging Activity of some Nigerian Medicinal Plants. *J. Pharm. Sci. & Pharm. Pract*. 8, 133-136.

Badmus, L. (2013). Beer and the Nations Economy – The Nigerian Telegraph, http://telegraphing.com/2013/II/beer-nations-economy. Retrieved 25-10-2014.

Bamforth, C.W. (1985). *J. of the Institute of Brewing*, *91*, 154.

Bamforth, C.W. (2006). Scientific Principles of Malting and Brewing: St Paul, Minesota: American Society of Brewing Chemists.

Bandyophadhay, U., Biswas, K., Chatterjee, R., Bandyyo, Padyay, D., Chattopadhyay, I., Ganguly, C.K., Chakroborty, T., Bhattacharya, K. and Banerjee, R.K. (2002). Gastroprotective effect of Neem (Azadirachta indica)

bark extract: Posisble involvement of H+-K+ - ATPase inhibition and scanvening of hydroxyl radical. *Life Sci*., 71:2845-2885.

Bradbury, M.G., Egan, S.W. and Bradbury, J.H. (1999). Determination of all forms of Cyanogens in Cassava roots and cassava products using picrate paper kits. *J. Sci. Food Agric.* 79:593-601.

Braide, V.P. (1991). Anthihepatotoxic Biochemical effects of kola viron, a Bifkvonoid of Garcinia kola seeds. *Phytotheraphy Res*. 5:35-37.

Briggs, D.E., Boulton, C.A., Brooks, P.A. and Stevens, R. (2004). Brewing: Science and Practice. Cambridge, England: Woodhead Publishing Limited.

Burgess, A.H. (1964). *Hops, Botany, Cultivation and Utilization*, Leonard Hill, London, 300.

Burkill, H.M. (1985). The useful plants of West Tropical Africa. 2nd Ed. Volume D, Families A-D. Royal Botanic Gardens, Kew, Richmond, United Kingdom, 960.

Busch, J. (1997). Home Brewery Advancement Step Mash for Customized Worts, Part I – The Enzymes that Breakdown Glucaus and Proteins. Brewing Technique 5 (4)

Butler, L.G. (1989). Effects of condensed tannins on animal nutrition in "Chemistry and Significance of condensed tannins" R.W. Hemingway and J.J. Karchegy. Eds. Pelnum Press, New York, pp. 391-402.

Buttler, G.W. and Bailey, R.W. (1973). Chemistry and Biochemistry of Herbage, Vol. 1, Academic Press, London and New York.

Carroll, T.C.N. (1979). *Technical Quarterly of the Master Brewers Association of America,* 16, 116.

Ceirwyn, S.J. (1999). Analytical Chemistry of Foods. An ASPEN Publication, Gaithersbury, Maryland, pp. 162 – 164.

Christian, G.D. (2004). Analytical Chemistry. Sixth Edition John Wiley and Sons, Danoyagani, New Delhi, pp. 574-603.

Clapperton, J.F. (1975). *European Brewing Convention Proceedings of the 15th Congress, Nice, 823.*

Comrie, A.A.D. (1967). "Brewing Water", *Brewer's Digest,* (42) 7.

Crombie,L. and Crombie, W.M.L. (1975). *Phytochemistry,* 14, 409.

Curhan, G.C. (1999). Epidemiologic evidence for the role of Oxalate in idiopathic nephrolithiasis. *J. Endourol.,* 13(9): 629-631.

Davis, R.H. (1991). Cyanogens. In: D'Mello JPF, Duffus C.M., Duffus J.H. (eds.). Toxic Substances in Crop Plants: Cambridge: *The Royal Society of Chemistry*.

De Clerck, J. and De Dijeker, G. (1957). *European Brewing Convention Proceeding of the 15th Congress, Copenhagen,* p. 43.

De Clerck, J. (1957). A Textbook of Brewing Volume 1, Translated by K. Barton-Wright. London: Chapman-Hall Ltd.

Dike, M.C. (2010). Proximate, Phytochemical and nutrient compositions of some fruits, seeds, and leaves of some plant species at Umudike, Nigeria. *ARPN Journal of Agricultural and Biological Sciences*, 5(1) 7-16.

Donaldin, G., Spalla, S. and Beone, G.M. (2008). "Arsenic, Cadmium and Lead in beers from the Italian Market." *J. Inst. Brew.,* 114(4):283-288.

Drake, V.J. (2010). Vanderbilt University http://www.vitamineher buniversity.com/images/spacer.gff Medical Centre.

Edman, J.W. and Forbes, R.M. (1977). Mineral bioavalability from Phytate containing foods. *Food Prod. Dev.* 11: 46-49.

Eisner, T. (1990). Chemical Prospecting: A call for Action. In: Borman, F.H. and S.R. Keller (Eds.), Ezology, Economics and Ethics: The Broken Circle. Yale University Press, New Haven, CT, pp: 105-110.

Eleyinmi, A.F., Sporns, P. and Bressler, D.C. (2008). Nutritional Composition of Gongronena latifolium and Vernonia amygdalina. *Nutrition and Food Science,* 38(2): 99-109.

Elzinga, K. (2005). "Beer". The Structure of American Industry, edited by Walter Adama and James Brock. Upper Saddle River, New Jessey: Pearson Hall.

Emeka, E.J.I. and Obiora, O. (2009). Effect of long term consumption of a diet supplemented with leaves of *Gongronema latifolium* Barth on some biochemical and histological parameters in male albino rats. *Journal of Biological Sciences,* 9(8): 859-865.

EPA(2009). Washington, D.C. Drinking Water Contaminants.

European Brewery Convention (1975). Analytica – EBC, 3rd Edn., Schweizer Brauerei-Rundschau, Zurich.

FAO/WHO (2011). Evaluation of Certain Contaminants in Food. Seventy Second report of the Joint FAO/WHO Expert Committee on Food Additives: Geneva, World Health Organization (WHO) Technical Report Series, No. 959.

FAOSTAT (2009). Food and Agricultural Organization of the United Nations. (http://foastat:fao.org/faostat) Retrieved 02-04-2011.

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

Fernie, A.R. and Schauer, N. (2008). Trends Genet. 25:39-48.

Finar, I.L. (1975). Organic Chemistry Volume 2: Stereochemistry and the Chemistry of Natural Products. Fifth Edition. Pearson Education, Ltd. Pataparganj, Delhi, India. pp. 60-64.

Fix, G. (1989). Beer Foam and CO_2 Levels. The New Brewer 6 (2).

Fix, G. and Laurie, F. (1997). An Analysis of Brewing Techniques. Boulder, Colerado; *Brewers Publications*.

Freeman, P.C. (1999). "Barley and Malting." The Practical Brewer, edited by John T. McCabe, Wauwatosa Wisconsin. *Master Brewers Association of America*.

Gail, E., Gos, S., Kulzer, R., Lorosch, J. Rubo, A. and Sauer, M. (2007). "Cayno compounds, Inorganic", *Ullman's Encyclopedia of Industrial Chemistry,* Weinheim: Wiley-VCH.

Georgieva, N., Yonkova, G., Ginova, T. and Terzi, A. (2007). Scientific Study and Research. Vol VIII (2) pp. 179-184.

Goldamer, T. (2008). *Brewers Handbook.* Apex Publishers, U.S.A.

Grant, H.L. (1979). *Journal of American Society of Brewing Chemist*, 27(2).

Grant, H.L. (1977). The Practical Brewer. Master Brewers' Association of the Americas. Impressions Inc. Madison, Wisconsin. 128-146.

Guisseppe, R. and T.M. Baratta (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Afr. J. Biotechnol*., 69(2): 167-174.

Gupta, S.C. (2011). Fundamentals of Statistics. Smith Revised and Enlarged Edition. Himalaya Publishing House, Delhi, Mumbia pp. 23.1-23.37.

Gutcho, M. (1976). Alcoholic Beveages Processes. London, UK: Noyes Press.

Hammer, K.A., Carson, C.F. and Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol*., 86(6), 985.

Haque, M.R., Bradbury, J.H. (2002). Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. Food Chemistry: 77(1): 107-114.

Harbone, J.B. (1995). Phytochemical Methods. London. Chapman and Hall Ltd. Pp 49-188.

Higdon, J. (2001). Vanderbilt University http://www.vitamineher buniversity.com/images/spacer.gff Medical Centre.

Hop Liaison Committee (1967). *J. Inst. Brewing 73*, 421.

Hough, J.S., Briggs, D.E., Stevens, R and Young, T.W. (1982). *Malting and Brewing Science*, Vol. 2, 2nd ed., Chapman and Hall, London England pp 389-452.

http://www.framalpha.com/inputs/?i=boiling+point+of+hydrogen+cyanide.
http//www.vitamineherbuniversity.com/topic/asp?category=2&topics

Hudson, J.R. (1970). *J. Inst. Brewing*, *75*, 164.

Hudson, J.R. (1997). Wort Boiling. "The Practical Brewer, edited by H.M. Broderick. Madison, Wisconsin: *Master* Brewers *Association of America*.

Igile, G.O., Oleszek, W., Burda, S., and Jurzysta M. (1995). Nutritional Assessment of Vernonia Amygdalina leaves in growing mice. *J. Agric Food Chem*. 43: 2162-2166.

Institute of Brewing (1977). Recommended Methods of Analysis. The Institute of Brewing, London.

Irvine, F.R. (1961). Wood Plants of Ghana, with special reference to their uses. Oxford University Press, London, United Kingdom. 868pp.

Iwu, M.M., O.A. Igboko, C.O. Okunji and M.S. Tempesta (1990). Antidiabetic and aldose reductase activities of Biflavanones of *Garcinia kola. J. Pharm. Pharmacol*. 42. 290-292.

Jackson, A.A. (2000). Advances in Experimental Medicine and Biology. Plenum Press, New York.

Jaffe, W. (1979). In I.E. Liener (Ed.): Toxic Constituents of Plant Foodstffs, Academic Press, Inc. New York, p. 69.

Joshi, B., Sah, G.P., Basnet, B.B., Bhatt, M.R., Sharma, D., Subedi, K., Pandey, J. and Malla, R. (2010). Phytochemical extraction of antimicrobial properties of different medicinal plants. *Ocimum sanctum* (Tulsi), Eugenia caryophllata (dove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem). *Journal of Microbiology and Antimicrobials* vol 3(1). pp 1-7.

Keay, R.W.J., Onochie, C.F.C. and Stanfield, D.P. (1964). Nigerian Trees, Nigerian Press Ltd, Lagos, Vol. I.P. 153 and Vol. 2 PP 25-434.

Kneen, E. (1994). A Comparative Study for the Development of Amylases of Germinated Cereals: *Cereal Chem* 21, 304-314.

Koller, H. (1969). *J. Inst. Brewing*, 75,175.

Kramer, P. (2006). "Barley, Malt and Malting Translated by Dr. Trevor Wainwright. Berlin, Germany: VLB Berlin.

Kraus-Weyerman, T. (1998). pH in the Brewery, A much underestimated Brewing Variable pp 1-30.

Kunze, W. (1999). *Hops and Hop Products*, 8th ed., Jaenicke Inc., USA pp 40-60.

Laws, D.R.J. (1981). Hops. *Brewer's Guardian* 1105: 71-74.

Liener, I.E. and Kakade, M.L. (1980). Protease Inhibitors. In: The Lectins: Properties, Functions and Applications in Biology and Medicine (Eds. I.E. Liener, N. Sharon and I.J. Goldstein). Academic Press, New York. Pp. 527-552.

Mabberley, D.J. (1995). *Azadirachta*. pp. 337-343. In: *Flora malesiana* Ser. Spermtophyta 12(1): 1-407.

MacDonald (1984). Current Approaches to Brewery Fermentations. Progress in Industrial Microbiology, edited by M.E. Bushell, Guildford, United Kingdom: *Elsevier*.

Mann, A.G., Tam, C.C., Higgins, C.D. and Lodrigues, L.C. (2007). The association between drinking water turbidity and gastrointestinal illness: a systematic review. BMC Public Health. 7(256):1-7.

McWilliam, I.C. and Philips, A.W. (1959). *Chem and Ind.*, p. 364.

Meilgaurrd, M.C, and Peppard, T.L. (1986). *The Flavour of beer*, In: Morton, I.D. and Maclead, A.J. (eds.). *Food flavours, Part B. The flavor of Beverages*, Elsevier, New York, 1986.

Micheal, M. (2004). Beer and Its Drinkers: An Ancient near Eastern Love Story. *Near Eastern Archaeology* 67(2): 84-95.

Moll, M.M. (1979). "Water in Malting and Brewing" Brewing Science. Volum 1, edited by J.R.A. Pollerk. London, United Kingdom. Academic Press.

Moncrieff, R.W. (1967). *The Chemical Senses,* 3rd edn. Leonard Hill, London.

National Agency for Food and Drug Administration and Control (NAFDAC) (2002). Regulations in Lager beers produced in Nigeria. National Agency for Food and Drug Administration and Control, Abuja, Nigeria.

Nelson, D.L. and Cox, M.M. (2005). Lehninger's Principles of Biochemistry (4th ed.) New York: WH Freeman.

Nelson, M. (2005). The Barbarian's Beverage: A History of Beer in Ancient Europe. Abingdon, Oxon: Routledge Press.

NIBREWNEWS (2012). Nigerian Breweries News, Issue No. 30 Vol. 40 and (2013) Issue No. 17 Vol. 33.

Nigerian Stock Exchange (2012). "Listed Companies on NSE." Retrieved August 31, 2012.

Nwanjo, H.U. and Alumanah E.O. (2006). Effect of aqueous extract of Gongronema latifolium on some indices of liver function in rats. *Global Journal of Medical Sciences* 5(1): 17-20.

O'Rourke, T. (1998). "The Treatment and use of Water in Brewing" *Brewers' Guardian*, 127(12).

Okafor, N. (2007)., *Modern Industrial Microbiology and Biotechnology:* Enfield (NH). Science Publishers.

Okafor, N. and Anichie, G. (1983). West African hop substitute for sorghum lager. *Brewing and Distillation International*, 13, 20-21.

Okolie, U.V., Okeke, C.E., Oli J.M. and Ehiemere, I.O. (2008). Hypogly caemic indices of Vemonia amygdalina on postpaandial blood glycose concentration of healthy humans. *African Journal of Biotechnology* 7(24) 481-4585.

Okpoko, P.O. (2010). The use of Bitter Leaft (*Vernonia Amygdalina)* Extract as a means of extending the Shelf-life of Locally Brewed Sorghum Beer. B.Sc. Project Department of Biochemistry, Faculty of Natural Sciences, Caritas University, Enugu.

Oliver-Bever, B. (1986). Mechanical Plants in Tropical West Africa. Cambridge University Press, Cambridge, United Kingdom 375pp.

Ologhobo, A.D. and Fetuga, B.L. (1983). Investigation on the trypsin inhibitor, heamagglutinin, phytic and tannin acid contents of cowpea (*Vigna unguiculalata).* Food Chem. 12:254.

Olsztyn, Igile, G.O., Oleszek, W., Jurzysta, M., Burda, S., Fafunso, M., and Fasanmade, A.A. (1994). Flavonoids from *Vernonia amygdalina* and their antioxidant activities. *J. Agric Food Chem*. 42:2445-2448.

Onwuka, C.F.I., Akinsoyinu, A.O. and Tewe, O.O. (1989). Feed Value of Some Nigerian browse plant: chemical composition and *in vitro* digestibility. *East African Agricultural and Forestry Journal* 54, pp. 157-163.

Onyeike, E.N. and Osuji, J.O. (2003). Research Techniques in Biological and Chemical Sciences. Springfield Publishers Limited, Owerri, Nigeria pp: 403.

Osagie, A.U., Muzquiz, M., Burbano, C.O., Cuadrado, C., Ayet, G., Castano, A. (1996). Some anti-nutritional constituents in ten staple food items grown in Nigeria. Trop. Sc. (U.K.), 36:109-115.

Palmer, G.H. (2006). "Barley and Malt". Handbook of Brewing, edited by Fergus G. Priest and Graham G. Stewart. Boca Raton, Florida: CRC Press, Taylor and Francis Group.

Parek, J., Jadeja, D. and Chanda, S. (2005). Efficacy of Aqueous and Methanol Extracts of some medicinal plants for Potential Antibacterial Activity. *Turk. J. Biol*., 29: 203-210.

Parker, W.E. and Richardson, P.J. (1970). *Journal of the Institute of Brewing*, 76, 191.

Patnaik, P. (2002). Handbook of Inorganic Chemicals. McGraw-Hill. ISBN 0-07-0494398.

Peacock, V. (2009). Hop Chemistry 101. SE Regional MBAA 35(1).

Pennington, T.D. and Style, B.T. (1975). A generic monograph of the Meliaceae. *Blumea* 22:419-540.

Rehberger, A.J. and Gary, E.L. (1995). “Brewing”. Handbook of Brewing, edited by William A. Hardwick. New York, Marcel Dekker Inc.

Russel, R.J., Kerry, P.S., Stevens, D.J., Stainhauer, D.A., Martin, S.R., Gamblm S.J., Skehel, J.J. (2000). *Proc. Natl. Acad.* Sci: US. 105 (46) 17736-41. Doi: 10.1073/pans.0807142105.PMC2584702. PMID19004788

Sanchez, G.W. (1999). “Water”. The Practical Brewer edited by John T. MccAbe. Wanwatosa Wisconsin: *Master Brewers Association of the Americas*.

Sharp, F.R. and Laws D.R.J. (1981). *Gongronema latifolium.* Proceedings of the Second National Seminar on Fruits and Vegetables Ibadan, 12-15.

Shellie, R.A., Poynter, S.D.H, Li, J., Gathercole, J.L., Whittock, S.P. and Koutoulis, A. (2009). Varietal characterization of hop (*Humulus lupulus* L.) by GC-MS analysis of hop cone extracts. *J. Sep. Sci.* 32, 3720-3725.

Siddiqui, A.A. and Ali, M. (1997). Practical Pharmaceutical Chemistry. 1st ed. CBS Publishers and Distributors, New Delhi, pp. 126-121.

Sidor, L. (2006). Hops and Preparation of Hops. Raw Materials and Brewhouse Operations. Volume 1, edited by Kari Ockert. St. Paul, Minnesota: *Master Brewers Association of America*.

Simeonova, F.P. and Fishbein, L. (2004). Hydrogen cyanide and cyanides: Human health aspects. Concise International Chemical Assessment Document 61. Geneva: World Health Organization.

Singh, N. and Sastri, M.S. (1981). Antimicrobial Activity of Neem oil. *Indian J. Pharmacol*., 13:102.

Sofowora, A. (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books, Ibadan.

Standard Organization of Nigeria (SON) (2003). Safe drinking water regulation. Standard Organization of Nigeria: Abuja, Nigeria.

Stewart, G.G. and Russel, I. (1985). Modern Brewing Biotechnology. “Food and Beverage Products, edited by Murray Moo-Young. Oxford, England, Pergamon.

Svendsen, R. and Lund, W. (2000). Speciation of Cu, Fe, and Mn in beer using ion exchange separation and size-exclusion chromatography in combination with Electrothermal atomic absorption spectroscopy.

Tegula, A. Tchoumbone, J., Mayaka, B.T. and Tnakou C.M. (1993). The growth of Broiler chickens as affected by the replacement of graded levels of maize by *Vernonia amygdalina* in the finisher diet. *Anim. Feed Sci. Technol*. 40, pp 233-237.

Tenney, R.I. (1985). Fermentation Symposium – Part 1 – Rationale of the Brewing Fermentation. MBAA Technical Quarterly 22(4).

Terashima, K., Takaya Y. and Niwa M. (2002). Powerful antioxidative agents based on garcinoic acid from *Garcinia kola: Bioorganic Med. Chem*., 10(5): 1619-1625.

Thomas, G.G. (1997). A.R. Wye College, P. 43

Thomas, G.G. and Neve, R.A. (1976). *J. Inst. Brewing 82*, 41.

Trease, G.E. and Evans, W.C. (1989). Pharmacognosy. 13th edn. Bailliere Tindall, London. Pp 176-180.

Ubuoh, E.A. (2013). Analysis of metal concentrations in selected canned beers consumed in Owerri Urban, Imo State, Nigeria; *Int. J. Chem. Mater*. Sci pp. 090-095.

Uko, E.E. (1988). Preliminary Studies on tropical hop substitutes. M.Sc. thesis. Department of Food Technology, Faculty of Technology, University of Ibadan.

UNMD (2000). Millennium Development Goal Reports. 47pp.

Usher, G. (1974). A dictionary of plants used by man. Constable and Co Ltd. London. 598pp.

Van Der Aar, P.C. (1995). Flocculation of Brewers' Lager Yeast. MBAA Technical Quarterly, 32 (222).

Verzele, M. and de Kenkeleire, D. (1991). Chemistry and Analysis of Hop *and* Bitter Acids, *Elsevier*, Amsterdam.

Vetter, J. (2000). "Plant Cyanogenic glycosides" *Toxicon 38* (1):11-36.

Viji, M.O. and Parvatham, R. (2011). Antimicrobial and cytotoci profile changes in leaf, stem, and root tissues of Withania somnifera-poshita variety. *Int. J. Pharmaceut*. Biomed Res. 2(3):81-89.

Wainwright, T. (1971). Biochemistry of Brewing. Modern Brewing Technology, edited by W.P.K. Findlay. Cleveland, Ohio: The Macmillan Press.

Wang, Z.N., Wang, M.Y. Mei, W.L., Hand, Z. and Dai, H.F. (2008). *A New cytotoric pregnanone from calotropis gigantea.* Molecules, Volume 12, Issue 12, pp. 3033-3039.

Westwood, K. (1994). Hop products and their effect on bittering quality. *Brewers' Guardian*, 123 (12).

World Health Organization (WHO) (1993). Evaluation of Certain food Additives and Contaminants. Technical Report Series No. 837. World Health Organization Geneva.

World Health Organization (WHO) (2001). Safety evaluation of certain food additives and contaminants: Cadmium. WHO Food additives series 46: Joint FAO/WHO Expert Committee on Food Additives.

Zurcher, C. and Kursawe (1973). *European Convention Proceedings of the 14th Congress*, Salzburg, 445.

APPENDICES

Appendix 1

Significance levels of taste-test results [48]

Number of tasters	Two-sample test no. of concurring choices necessary to establish the significance of a result as indicated by			Triangular test: Difference test no. of correct answers necessary to establish the significance of a result as indicated by			Triangular test: Quality or preference test no. of concurring choices by tasters selecting correctly to establish the significance of a result as indicated by			Number of tasters
	*	**	***	*	**	***	*	**	***	
1	—	—	—	—	—	—	—	—	—	1
2	—	—	—	—	—	—	—	—	—	2
3	—	—	—	3	—	—	3	3	—	3
4	—	—	—	4	—	—	3	4	—	4
5	—	—	—	4	5	—	4	4	5	5
6	6	—	—	5	6	—	4	5	6	6
7	7	—	—	5	6	7	4	5	6	7
8	8	8	—	6	7	8	5	5	6	8
9	8	9	—	6	7	8	5	6	7	9
10	9	10	—	7	8	9	5	6	7	10
11	10	11	11	7	8	10	5	6	8	11
12	10	11	12	8	9	10	6	7	8	12
13	11	12	13	8	9	11	6	7	8	13
14	12	13	14	9	10	11	6	7	9	14
15	12	13	14	9	10	12	7	8	9	15
16	13	14	15	9	11	12	7	8	9	16
17	13	15	16	10	11	13	7	8	10	17
18	14	15	17	10	12	13	7	9	10	18
19	15	16	17	11	12	14	8	9	10	19
20	15	17	18	11	13	14	8	9	11	20
21	16	17	19	12	13	15	8	9	11	21
22	17	18	19	12	14	15	8	10	11	22
23	17	19	20	12	14	16	9	10	12	23
24	18	19	21	13	15	16	9	10	12	24
25	18	20	21	13	15	17	9	10	12	25
26	19	20	22	14	15	17	9	11	12	26
27	20	21	23	14	16	18	10	11	13	27
28	20	22	23	15	16	18	10	11	13	28
29	21	22	27	15	17	19	10	11	13	29
30	21	23	25	15	17	19	10	12	13	30
31	22	24	25	16	18	20	10	12	14	31
32	23	24	26	16	18	20	11	12	14	32
33	23	25	27	17	18	21	11	13	14	33
34	24	25	27	17	19	21	11	13	15	34
35	24	26	28	17	19	22	11	13	15	35
36	25	27	29	18	20	22	12	13	15	36
37	25	27	29	18	20	22	12	14	15	37
38	26	28	30	19	21	23	12	14	16	38
39	27	28	31	19	21	23	12	14	16	39
40	27	29	31	19	21	24	13	14	16	40
41	27	29	32	20	22	24	13†	14†	16†	41
42	28	30	32	20	22	25	13†	14	16†	42
43	28	30	33	21	23	25	13†	14	16	43
44	29	31	33	21	23	25	13	14	16	44
45	30	32	34	22	24	26	13	14	16	45
46	30	32	35	22	24	26	13	15	16	46
47	31	33	35	23	24	27	13	15	17	47
48	31	33	36	23	25	23	14	15	17	48
49	32	34	37	23	25	28	14	15	17	49
50	32	35	37	24	26	28	14	16	18	50
51	33	35	38	24	26	29	14	16	18	51
52	34	36	38	24	27	29	14	16	18	52
53	34	36	39	25	27	29	15	16	18	53
54	35	37	40	25	27	30	15	17	19	54
55	35	38	40	26	28	30	15	17	19	55
56	36	38	41	26	28	31	15	17	19	56
57	36	39	41	26	29	31	16	17	19	57
58	37	39	42	27	29	32	16	17	20	58
59	38	40	43	27	29	32	16	18	20	59
60	38	40	43	28	30	33	16	18	20	60
61	39	41	44	28	30	33	16	18	20	61
62	39	42	44	28	31	33	17	18	20	62
63	40	42	45	29	31	34	17	19	21	63
64	40	43	46	29	32	34	17	19	21	64
65	41	43	46	30	32	35	17	19	21	65
66	41	44	47	30	32	35	17	19	21	66
67	42	45	47	30	33	36	18	20	22	67

† Calculation by the χ^2 method gives a lower number, but adjustment has been made to conform with values obtained by binomial expansion. 1, 2 and 3 stars represent significance at the 5%, 1% and 0·1% levels respectively.