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Bitterleaf as local substitute for hops in the nigerian brewing industry

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ABSTRACT

*A lot of effort had been made in the Nigerian brewing industry to substitute barley with some local cereals in production of alcoholic and non-alcoholic drinks. However, the substitution of hops with local raw materials has not received commensurate attention. This paper investigated the brewing qualities of bitterleaf (*Vernonia Amygdalina*) using extraction with appropriate solvent and steam or hydro distillation to obtain the bitterleaf extract. Thereafter, the extract was characterized in order to determine its suitability for use in brewing by comparing it with those of standard commercial hops values. The physicochemical analysis of the bitterleaf showed that the sample extract had brewing properties or variables as follows: Iso-alpha acid (mg/l) of 8.52, alpha acid (mg/l) of 9.27, total resin (%) of 20.4, essential oil (%) of 1.20 and fat content (%) of 7.00. The Analytical Bitterness Unit (ABU) was found to be adequate and was equal to 8.73 European Bitterness Unit (EBU). The properties or variables of the present bitterleaf extract was found to compare favourable with those of the commercial hops used as basis for comparison though with variation in the fat content which was found to be 7.00% compared to the commercial hop which was 3.12%. Consequently, the results obtained showed that bitterleaf presents a potential substitute for hops in the Nigerian brewing industry though the timing of its use will depend upon economic and political considerations and the supply and demand for bitterleaf and hops.*

INTRODUCTION

Hops (*Humulus lupulus*)

Hops (*humulus lupulus*) are rough-stemmed, twinning perennial herb of the melbery family, native to Europe and Asia and widely naturalized in North America. The hop plant is a dioecious perennial species, and only female cones are used for beer brewing. Hops are used to impart bitterness, flavor, colour, foam, stability, antiseptic and preservative properties to modern beers as established worldwide^{1,2,3}.

Although there is only one hop species (*Humulus lupulus*) that is used for brewing beer, there are a number of varieties in that species, each with its own spectrum of characteristics⁴. Varieties of hops are chosen for the properties of bitterness, flavor, or bouquet that they will lend to the beer⁵. Hop varieties can be roughly divided into bittering hops and aroma hops though there are hop that can be considered dual-purpose⁶.

Bittering hop varieties are those that impart bitter flavor to beer and have high alpha acid levels. They usually have a high alpha acid content. Aroma hops have low-to-medium alpha levels and mainly impart characteristics hops aroma to beer. Dual-purpose hops tend to have intermediate level of alpha acids together with desirable aroma properties. Hops, though selected and bred for the bitter and aromatic qualities they impart to beer, the female flowers or cones produce tiny gland that contains chemicals of value in brewing^{6,7,8}. Their brewing value comes from the resins and essential oils found in the lupulin glands^{9, 10}. This gives the 'character' of the hop. Other constituents of hops include varying amounts of tannins, carbohydrates, protein, lipids and waxes. However, the most important constituents are the hop resins from which are derived the principal bitter substances in beer and other non-alcoholic beverage and the oil which is responsible for hop character.

Thomas Hofmann *et. al*¹¹ at the Technical University of Munich sought to identify what causes the bitter, off-taste in old beer. The research uncovered fifty six substances that contributed to the bitter taste of beer. These are mostly "prenylated polyketides" derived from hops. From this list, it was discovered that five substances are largely responsible for the harsh taste of ageing beer. It is believed that the study offered the scientific basis for a knowledge-based extension of the shelf-life of hops-derived beers. Furthermore, the research was aimed at using these discovered compounds as analytical marker molecules in order to monitor taste development during industrial brewing processes or aging studies on a molecular level. Thus, the aim is to control and tailor industrial processes based on knowledge rather than on trial and error.

Shelf-life of beers could be extended by controlling the initial pH of the beer or the maintenance of low temperatures during storage^{12, 13}. In addition, the use of iso-hops which contain only the stable *cis*-iso-alpha acids instead of the instable *trans*-iso-alpha acids could help extend shelf life significantly^{14, 15, 16}.

However, hop plant is a temperate crop and cannot be successfully grown in a tropical country like Nigeria; hence its importation for brewing in Nigeria is imperative.

Bitterleaf (*Vernonia Amygdalina*)

As a result of the growing trend towards sourcing of local substitute for industrial raw materials in Nigeria, a lot of efforts have been made in the brewing industry for the substitution of barley with some local cereals. However, the substitution of hops with local raw materials has not received commensurate attention.

Bitterleaf (*Vernonia amygdalina*), commonly called Ewuro and Etidot in Oyo and Cross-River states of Nigeria is a shrub or small tree of 2-5m with petiolate leaf of about 6mm diameter and elliptic shape. The leaves are green with a characteristics odour and a better taste¹⁷. No seeds are produced and the tree has therefore to be distributed through the cutting¹⁷. It grows under a range

of ecological zones in Africa and produces large mass of forage and is drought tolerant¹⁸. There are about 200 species of vernonia¹⁹. The leaves are used for human consumption and washed before eating to get rid of the bitter taste. They are used as vegetable and stimulate the digestive system, as well as reduce fever. Furthermore, they are used as local medicine against leech which transmits bilharzias. It can also be used, instead of hops in making beer¹⁷ and also found in homes in villages as fence post and post-herb¹⁷. It is a small shrub that grows predominantly in tropical Africa²⁰. In Nigeria, the plant is locally called bitterleaf because of its bitter taste. The leaves, especially the young ones are eaten and being a vegetable, it is usually nurtured as annuals though a few could be grown as perennials.

Generally, it has been found that vernonia amygdalina have an astringent taste, which affects its intake¹⁷. The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins, and glycosides[21-26,18]. Studies have also reported the antiplasmodial effects of some sesquiterpene and steroidal constituents of bitterleaf which are important constituents of brewing requirements [26-34]. Also, antioxidant activities of luteolin, leteccin 7.0, a-glueuronosid and luteolin 7.0 and a-glueoside flavonoid compounds have been isolated from the leaves of bitterleaf using coupled oxidation of a-carotene linoleci acid[21,35,36,37,38]. Some peptides were also isolated from the aqueous extract of vernonia amygdalina³⁹. The constituents of the peptides were shown as potent inhibitors of nitrogen activated kinases crucial for tumor growth in human. Anti-nutritional factors in bitterleaf include tannins (as tannic acid), saponins, glycosides and alkaloids.

Additionally, studies have shown that the leaves of the tropical vegetable, grogonema latifolium (utazi) is of great potential as local substitute for hops. It was found that the plant possessed some antiseptic properties against the micro-organism responsible for the deterioration of alcoholic and non-alcoholic beverages. The chemical properties of beer brewed using this plant did not differ much from that brewed with hop though their organoleptic differences were pronounced³⁷. However, the physicochemical properties of the extract were not characterized as it was only used for brewing and sensory analysis.

Aims and Objectives of the Research

This work investigated the brewing qualities of bitterleaf using extraction and steam distillation to obtain the extract and subsequent characterization to determine some physicochemical properties or variables of the bitterleaf extract which were then compared to those of commercial hop values in use taking into consideration other competitive uses of bitterleaf.

MATERIALS AND METHODS

The experimental procedures involved the procurement of materials and equipment; pretreatment of raw bitterleaf; steam distillation and extraction of the extract from the bitterleaf and characterization of the bitterleaf extract obtained. A step-by-step procedure was followed in the preparation of the bitterleaf extract so as to provide for the desired particle size free of moisture and foreign particles.

In this work, only the unconventional materials and major equipment set-up used in the experiment are given in Tables 1 and 2 respectively.

Table 1: List of Material Used for the Experiment

Materials	Source	Research Code Name	Comments
Bitterleaf	Central Market Minna, Niger State Railway Station Market, Kaduna, Kaduna State and Kuje Market, Abuja, F.C.T	-	Fresh greenish to dark blue in colour
Granulated Bitterleaf sample	Bitterleaf	-	Amber, dark brown in colour

Table 2: List of Equipment Used for the Experiment

Materials	Source	Model	Comments
Steam extraction still	Awal Industries Kaduna	NA	Stainless steel material
Separating funnels and settling tanks	AG Borosilicate, England	BS 2021	Glass apparatus
Connecting slits	Quickfit, England	DA 23	Glass apparatus
Weighing Balance	Ohaus, USA	B 300D	Digital display
Measuring cylinder	Technico, England	BS 604	Digital display
Condensers	AG, Borosilicate, England	BS 1848	Glass apparatus
Oven	Gallenkamp, England	CE 94	Vacuum drier
Stack of sieves	Chemical Engineering Laboratory, FUT, Minna	SOS 241	Stainless steel

Procurement and Pretreatment of Raw Bitterleaf

The raw bitterleaf used in this study was sourced freshly from Minna Central Market in Niger State, Railway Station Market in Kaduna State and Kuje market in Abuja, all in Nigeria respectively at two different periods. The leaves were thoroughly washed and screened to remove foreign bodies. They were then dried at ambient temperature (25⁰C) for five days to eliminate moisture. The dried leaves were therefore crushed and pounded using a mortar and a pestle into particle size of 0.75mm and below. The size reduction was done in order to increase the surface area for contact with the solvent because the particle of a soluble material is surrounded by a matrix of insoluble matter and thus, the size reduction will allow the solvent to penetrate and diffuse into the particle to allow the extract to diffuse out accordingly.

Experimental Procedure

Preparation of bitter leaf extracts (Direct Extraction)

In the process, 20 grams of the granulated sample of particle size 0.75mm was measured into a round bottom flask which contained 200ml of distilled water. The mixture was rigorously agitated by swirling the flask. A reflux condenser was mounted and fitted onto the conical flask. The condenser was then connected to a tap water source. The vent of the flask was made air-tight to prevent the escape of the evaporating steam. The set-up was held tight with a retort-stand and the mixture placed on an electric heater and the thermostat adjusted to maintain a constant heating rate at the boiling point of water (100⁰ C). The mixture was allowed to boil for the extraction time of 120 minutes. The vapour from the boiling solvent was made to condense and return to the mixture by means of a reflux condenser which was mounted on the flask through which water was constantly flowing. After the extraction had been completed, the heater was switched off, the solution allowed to cooled and afterward filtered using a filter paper placed on a funnel in a beaker. This procedure was repeated several times using fresh samples of same

mass to obtain sufficient quantity of the extract for analysis. The extract was used for Analytical Bitterness Level and Iso-alpha acid determinations.

Steam Distillation of Bitterleaf Sample for Essential Oil Determination

Twenty (20) grams of the granulated sample of particle size 0.75mm and 1500ml of water for steam generation was used at moderate heating rate. 1500ml of water was introduced into the bottom chamber of the still. The chamber was covered with a perforated metal plate in which a white filter cloth was placed. 20 grams of the granulated sample of particle size 0.75mm was then placed on the filter cloth. This was further covered with white filter cloth. The last perforated metal plate was placed on the top compartment. Finally, the still was made air-tight with the last covering to prevent the escape of the steam-oil mixture during heating. The set-up was then connected to a condenser via a pipe fixed at the top of the extraction still where an opening had been made. The delivery tube from the condenser was connected to the separating funnel to receive the mixture of steam and oil on condensation. The set-up was then mounted and connected to the heating source for extraction time of 120 minutes. At the end of the time interval, the set-up was switched-off and allowed to cool. The water-oil mixture was decanted to separate the oil from the water at the water-oil interface. Thereafter, the mass of the sample after extraction and drying in an electric oven was collected in a sample bottle and its mass recorded. This procedure was repeated several times to obtain sufficient quantity of the essential oil.

Indirect Extraction of Bitterleaf Sample for Fat Content Determination

In this process, 20 grams of the granulated sample of particle size 0.75mm was placed inside a thimble and inserted into the inner tube of the soxhlet extractor. This apparatus was then fitted to a round bottom flask which contained 200ml of n-hexane. A reflux condenser was also mounted and fitted on the apparatus. The set-up was held tight to a retort-stand and then placed on a heating mantle that was switched on for extraction time of 120 minutes at the boiling point of the solvent (n-hexane 60⁰). The vapour passed up through the tube, condensed by the condenser and the condensed solvent falls into the thimble and slowly fills the body of the soxhlet. When the solvent reached the top of the tube, it siphoned over into the flask and thus removed the portion of the sample that has been extracted in the thimble. The process repeated itself automatically for the extraction time of 120 minutes and the apparatus was dismantled.

The solvent recovery process involved using the same soxhlet extractor. The mixture of solvent and oil (also called miscella or extract) was heated in the flask. On constant heating, the solvent evaporated and thereafter, condensed in the thimble chamber. The solvent was collected before it siphoned back into the flask. The extracted oil was then recovered and the mass recorded. This procedure was repeated several times to obtain sufficient quantity of the extracted oil.

Determination of Total Resin

In this process, 20 grams of the granulated bitter leaf sample was dissolved in 100ml of cold methanol in a conical bottom flask and the mixture was vigorously agitated by swirling the flask. Thereafter, the solution was filtered. The filtrate containing the resin was then dried to a constant weight over water bath at 50⁰C. The total resin was then calculated as a percentage of the original sample weight (Hough *et al*, 1983 and Lob, 1977 methods).

Determination of Alpha Acid

Twenty (20) grams of the granulated bitterleaf sample was added to 100ml of cold methanol in a conical flask and the mixture was thoroughly agitated. The resulting solution was then centrifuged at 2,500 radiant per minute for 20 minutes and the extract decanted. The extract was then acidified with 10ml of 0.002N HCl and its absorbance read at 355nm, 325nm and 275nm respectively using a spectrophotometer. The spectrophotometer was switched on and calibrated using a blank (pure methanol). The sample was then inserted into the curette and the absorbance read at 275nm and recorded. The curette was then rinsed. The procedure was repeated and the absorbance read at 325nm and 355nm respectively. The alpha acid was then computed (Hough et. al, 1983 and Lob, 1977 methods).

Determination of Iso-Alpha Acid

Fifteen (15) mililiters of sample water extract obtained from direct extraction of the bitter leaf extract was acidified with 0.5ml of 6N HCl and mixed with 15ml of pure iso-octane in a shaker. 10ml of the iso-octane extract was then washed with 10ml of a mixture of methanol and 4N HCl which is methanol dissolved in 32ml of 4N HCl. Subsequently, 5ml of the washed iso-octane layer was diluted with 5ml of alkaline methanol and its absorbance read at 255nm. The iso-alpha acid (mg/l) was then computed.

Determination of Analytical Bitterness Level

Five (5) mililiters of the extract obtained from direct extraction of the bitterleaf extract was acidified with 0.5ml of 6N HCl. This was subsequently extracted with 10ml of Iso-octane in a shaker. The mixture was then agitated for 20 minutes and thereafter centrifuged for 10 minutes. The absorbance of the iso-octane extract was then determine at 275nm using a spectrophotometer. The analytical bitterness level was then computed.

RESULTS

Table 3.0: BitterLeaf Brewing Properties/Variables

Brewing variables	Values
Iso-alpha acid (mg/l)	8.52
Alpha acid (mg/l)	9.27
Total resin (%)	20.4
Essential oil (%)	1.20
Fat content (%)	7.00
Analytical Bitterness Unit =	8.73
European Bitterness Unit (EBU)	

Table 4.0: Commercial Hop (Lupo Fresh Brand) Properties/Variables

Brewing Properties/variables	Values
Essential oil (%)	1.14
Total resin (%)	19.53
Alpha acid (mg/L)	11.51
Iso-alpha acid (mg/L)	10.35
Fat content (%)	3.12

Source: Journal of Agricultural Technology, 1993

The results of the various analysis conducted is shown in Table 3.0 while standard commercial hops value for those analysis using the Lupo fresh brand constituents as basis for comparism is shown in Table 4.0. Also, Table 5.0 gives the range of the important brewing qualities of hop.

Table 5.0: Important Brewing Qualities of Hops and their Range of Values

Components	Range of Values
Resins	14 – 21%
Oil Content	0.5 – 1.5%

Source: Wolfgang Kunzi, 1999

RESULTS AND DISCUSSION

The research investigated the brewing parameters and qualities of bitterleaf (*Vernonia Amygdalina*) as a potential substitute for imported hops used in brewing operation for alcoholic and non-alcoholic beverages with respect to the key variables identified as necessary in hops. In this research, experiment and characterization of the physicochemical properties of the bitterleaf extract was conducted at three locations of Minna, Kaduna and Abuja to determine the influence of sourcing of the bitterleaf and this was found to be of no effect or relevance though it was important to evaluate the overall or average qualities from different location. The comparism was made with reference to those of standard commercial hops; Lupo fresh brand. The results are presented in Tables 3.0 to 4.0 while Table 5.0 gives a range of values for the two major brewing qualities of hop which formed the basis for which bitterleaf are compared.

From Table 3.0 and 4.0, comparing the various parameters, it was observed that the result obtained for iso-alpha acid content, alpha acid content, total resin and essential oil were found to be comparable to those of the standard hops value in Table 3.0. The essential oil content of the bitterleaf fell within the range of hop oil content from literature which was 0.5-1.5%. Similarly, the value of the bitterleaf resin fell within the range of 14-21% as obtained from literature. These are shown in Table 5.0.

However, the analyzed bitterleaf extract had a fat content of 7.0%. This was found to be on the high side when compared to the commercial hop whose value was 3.12% in Table 4.0. From literature, a high fat content was not desirable as it could lead to poor foam head stability particularly in the brewing of alcoholic drinks. The analytical bitterness unit was found to be very good at 8.73 EBU when compared to that of hops and from literature. This was attributed to the high bitterness level of bitterleaf as one of its major active principle.

Based on the comparison, it was observed that the brewing qualities of hops are present in bitterleaf to an appreciable level with variation in the fat content only. Thus, the quality of the bitterleaf extract from the analysis carried out can be said to be good since there were close similarities in properties of the standard commercial hops and the bitterleaf properties.

CONCLUSION

The conclusions emerging from this research indicated that bitterleaf (*Vernonia Amygdalina*) could be used as a hop substitutes in the brewing industry. The various important brewing

qualities of hops were tested for in bitterleaf and their values found to compare favourably with those of commercial hops, in this case, Lupo fresh brand which was used as the standard.

Though the fat content of bitterleaf was considered high in comparison to the commercial hops, this could further be investigated or blended with hops in order to create a basis for use of local raw materials in order to reduce the quantity of hops imported annually and thus save the much needed foreign exchange while encouraging local enterprises and initiatives.

Hence, it can be safely concluded that bitterleaf presents a potential substitute for hops in the brewing industry.

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