Comparative Analysis of the Essential Oil Contents of *Azadrichtaindica* and *Humuluslulpulus*: Could *Azadrichtaindica*substitute *Humuluslulpulus* in beer brewing?

ABSTRACT

Hops(*Humuluslulpulus*) give beer its brewing qualities (iso-alpha acid and essential oil). Hop acid is responsible for bitterness while hop essential oil is responsible for aroma and palate fullness. The aim of the study is to investigate the potentiality of substituting hop oil with neem(*Azadrichtaindica*) oil in beer brewing. The oils were analyzed using Fourier-Transform Infrared (FTIR)spectroscopic technique after extracting the oil by solvent extraction. Hydrocarbon and Sulphur contents were also investigated using their standard methods. FTIR results show that both oils contain similar functional groups. It was observed that some hydrocarbons (myrcene, ocimene and α and β-pinene) present in essential oil of hop were also present in neem leaf oil**.** Volatile sulphur compounds such as dimethyl sulphide, dimethyl disulphide and dimethyltrisulphide present in hop oil were also found in neem oil. This study revealed that neem oil could be used as a substitute for hop oil in aroma enhancement in beer.

Keywords: *Azadrichtaindica*, *Humuluslulpulus*, Essential oil, spectroscopic method, beer brewing

INTRODUCTION

A specific raw material is used to make every industrial product. Plants are a significant supplier of raw materials for many industrial products, including apparel, footwear, and a wide range of other items[1]. Additionally, plants serve as a source of rawmaterials for the production of biofuels[2], dyes[3], fragrances [4]insecticides [5, 6], medicines [7], and beverages like beer [8-10].

Beer makes up around 60% of the entire volume of the world's alcoholic beverage market, making it the most popular alcoholic beverage overall. It is frequently consumed at gatherings for friends, such as sporting events, concerts, and house parties. As a result of this, there are many smaller companies vying for market share among the highly competitive global players in the beer industry. To satisfy consumer desire for new options, a rising variety of craft beers and beer-mixed cocktails have emerged over the past ten years. Non-alcoholic beer is also becoming more popular, which can be ascribed to a portion of beer drinkers who prioritize their health and want to prevent any harmful consequences of alcohol while still desiring the sensation of drinking a beer [11, 12].

Due to the large number of young people in Nigeria who have access to money, beer consumption has significantly increased recently [13, 14]. Additionally, beer makes up 55% ($3.75 billion) of Nigeria's $6.5 billion alcohol market, followed by spirits (30%), and wines (15%). Statista [15] in 2021 estimates that ‘‘beer sales will generate $4.57 billion in revenue in 2021 (i.e., 2.2 trillion at 440/$1). That represents almost 12% of Nigeria's 17 trillion naira 2022 budget’’. Additionally, between 2022 and 2025, the market is anticipated to expand at a cumulative annual growth rate (CAGR) of 16.45%.

According to a new analysis by the Worldwide Brewing Alliance (WBA), the beer industry had a $2.2 billion impact on the Nigerian economy in 2019.The WBA stated during a webinar that the report, which was created by Oxford Economics, revealed the sector's impact on various economies.According to the paper, Nigeria's economy benefits from the domestic, international, and global supply chains of the global beer industry.The worldwide beer industry supported a $2.2 billion gross value-added contribution to Nigeria's GDP in 2019. This was the same as 0.6% of the country's GDP, or 73% of Zaria's economy [16]. As a result, a sizable share of imports into the nation have continued to be hops, which are needed to satisfy the demands of the already flourishing brewing industry. The main ingredients used to make beer are malt, hops, water, and yeast. The brewing industry depends on hop plants because some of its special compounds are very vital in the nutraceutical sector[17, 18]and today, the industry uses as much as 98% of hop crops produced worldwide [19].

Hops are used for their ability to bitter taste, flavour, and enhance aroma in beer. Hops are made up of essential oils and acids (α and β acids). In contrast to the acids, which are used for bittering and improving foam stability as well as acting as an antibiotic against microorganisms, hop's essential oils are used to enhance flavours and aromas[20 – 22].

The quality of hops and beer is currently only partially studied in the literature even though, the substitution of hops with Nigerian bitter vegetables abounds in literature [21 – 25]. There hasn't been any research that used the identical experimental setup and also took into account both of these factors and the substitution of hop essential oil with*A. indica* oil. In the current study, the impact of fragrance improvement on beers infused with A. indica oil using a multidisciplinary approach for characterization, i.e., FTIR, GC/MS, and GC/FID was examined for the first time.

The aim of this research is to study comparatively the essential oils of hop and *A. indica* in order to establish the potentiality of substituting the hop oil with that of *A. indica* in tropical beer brewing. The objectives are (a) to extract essential oil from *A. indica*leaves (b)to analyze the oil extracts for functional group analysis using FTIR, (c) to determine the hydrocarbon content of the oil extracts using GC/MS, (d) to determine the sulphur content of the oil extracts GCFID.The substitution of hops with *A. indica*in beer brewing in Nigeria would not only save Nigeria's hard-earned foreign exchange since Nigeria's brewing industry won't need to import hops but would give our local farmers jobs in the Agricultural sector.

MATERIALS AND METHODS

Extraction of Essential oil from *A. indica*

The leaves of *A.indica* used for the research were obtained in the herbarium of Nnamdi Azikiwe University, Awka, Anambra State and were identified by a taxonomist. The leaves were dried after which they were pulverized into powder using amanual grinder. A 352g of the pulverized powder was placed into the thimble and placed in the Soxhlet chamber containing 500mL N-hexane.After completing the extraction process, the solvent and extractor were placed ina water bath to evaporate the solvent and the oil was collected.

Sample Preparation for FTIR Analysis

We employed a protocol used by Ezea *et al*. [26] in the preparation of the oil for FTIR analysis. To get spectra heights, 0.5 mL of the oil was combined with 0.5 g of Kbr powder, and 1 mL of

Nujol was pipetted into the mixture to create a paste. This paste was then placed into the instrument sample mould and allowed to scan at wavelengths between 600 cm^{-1} and 4000 cm^{-1}.

GC-MS and GC-FID analysis

The method adopted by some researchers in the determination of hydrocarbon and volatilesulphur compounds [27 – 29]was used in this work. Briefly,gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) were carried out using an Agilent 6890N gas chromatograph and an Agilent 5973N mass selective detector (70 eV). Helium was used as the carrier gas, and its flow rate was 1000 L/min. A sample volume of 1 L (1% solution) was injected. The range of the scanning frequency was 50 to 550 m/z. A capillary column made of HP-5MS (30 m x 0.25 mm x 0.25 m) was used. The column temperature was first set at 50°C for 2 minutes, then increased to 150°C at 2 degrees per minute for 2 minutes, held for 2 minutes, and then increased to 250°C at 10 degrees per minute for 5 minutes and a homologous sequence of n-alkanes (C_8-C_{40}) were used.

RESULTS AND DISCUSSION

Table 1 presents all characteristic spectra observed in the sample for essential oils of *A. indica*as well as specific functional groups assigned to particular vibration spectra. *A. indica* essential oil displays very intense and distinct bands, which can be attributed to specific vibrations of appropriate functional groups corresponding in turn to the components contained therein. Essential oils are mixtures of various compounds, for example, carvone, limonene, pinene, cymol, terpenic alcohols, menthol and menthone with various isomers, piperitone, methyl acetate, and germacrene. In many studies, the authors have made appropriate adjustments to specific bands in the spectra of essential oils of plant origin of various species [30 -33] regarding specific vibrations in molecules or their moieties. However, many bands are difficult to properly assign to a specific functional group which may correspond to the content of a given amount of different substances (the reason is the fact that the substances present in particular oils often have similar chemical compositions). Table 1 presents in detail the frequencies of the characteristic spectra, along with the major extensions of the respective bands in the spectra of the essential oil sample, andtheir assignment to the appropriate functional group. The bottom indices also show the intensity of observed bands in typical spectra in the IR region.

The results of hydrocarbon content in both hop and neem oils indicated that some hydrocarbons (myrcene, ocimene and α & β-pinene) present in theessential oil of hop were also present in *A. indica* essential oil while humulene and caryophyllene were only present in the essential oil of hop but absent in *A. indica*. As secondary metabolites, essential oil components are influenced by environmental (climatic and soil) factors and plant organs [34 – 39] and this may explain the reasonfor the discrepancies in the composition of essential oil in *A. indica* and hops. The result however was in partial agreement with the findings of Dieckmann and Palamand [40].

Table 1: Result of FTIR spectra of *Azadiratchaindica* oil

S/N	Wavelength(cm^{-1})	Functional group	Compounds
1	752.3616	C-Br	Bromo compound C-Br stretch
2	842.7057	C-CI	Aliphatic Chloro compound C-CI stretch
3	1025.068	C-O-C	Ether CO symmetric stretch
4	1378.737	H_2C-CH_3	Ethane CH symmetric stretch
5	1477.960	H-CW	Ethene C=C anti-symmetric stretch
6	1620.787	R-$COONH_2$	Amide CN stretch
7	1838.131	R-COOR	Cyclic ester COO stretch
8	2058.256	RCOO	Carboxylic acid CO stretch
9	2226.013	R_2C=O	Carbonyl C=0 anti- symmetric stretch
10	2294.906	R_2C-O	Carbonyl C=0 anti-symmetric stretch'
11	2448.348	R-C=N	Nitrile CN anti-symmetric stretch
12	2524.535	CH_2SH	Thiol SH stretch
13	2766.888	CH_2	Methylene CH symmetric stretch
14	2949.748	R-S-ON	Thiocyanate SCN antisymmetric stretch
15	3043.655	RCHOH	1° alcohol OH stretch

16	3197.924	RCHOH	1° alcohol OH stretch
17	3444.58	$R-NH_2$	2 amine N-H bend
18	3695.667	R_3N	3 amine N-H bend
19	381 1.956	R_3CHOH	Phenol OH symmetric stretch

Volatile sulphur compounds such as dimethyl sulphide, dimethyl disulphide and dimethyl trisulphide present in hop oil were also found in *A. indica* oil while 3-(methylthio) propanal, 3-(methylthio) propanol, 3-(methylthio) propyl acetate, ethyl 3-(methylthio)-propanoate, dihydro-2-methyl-3(2H)-thiophenone, 2-thiophene-carboxyaldehyde, 5-methyl-2-thiophenecarboxaldehyde, benzothiophene and benzothiazole were present in hops only [20]. Therefore, in terms of volatile sulphur compounds, *A. indica* oil could not be a good alternative for hops in beer brewing.

CONCLUSION

FTIR results show that both oils contain similar functional groups. Myrcene, ocimene, α and β-pinene were present in both theessential oils of hop and *A. indica*oil**.** Sulphur compounds such as dimethyl sulphide, dimethyl disulphide and dimethyltrisulphide present in hop oil were also found in *A. indica* oil. This study revealed that the essential oil of *A. indica* possessed promising potential in aroma enhancement in tropical beer brewing. Consequently, it is recommended that beer brewing industries may consider the use of *A. indica*as apossible hop substitute.

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