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Determination of the Flavoring Components in *Vitex doniana* Fruit Following Hydrodistillation Extraction

Imoisi Chinyere[1,*], Iyasele U. Julius[1], Okhale E. Samuel[2]

[1]Department of Chemistry, University of Benin, Benin City, Nigeria
[2]Departments of Medicinal Plant Research and Traditional Medicine,
National Institute for Pharmaceutical Research and Development (NIPRD), Garki, Abuja, Nigeria
*Corresponding author: imoisi.chinyere@gmail.com

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Abstract In traditional medicine and aromatherapy, use of essential oils and their flavor compounds have been known for the management of various human diseases. The flavor structures of black plum (*Vitex doniana*) sweet fruit are unavailable though widely eaten by natives. Therefore, this research is aimed at using spectroscopic techniques to identify the chemical structure of the specific flavor in the syrup responsible for its unique aroma and taste. Hydrodistillation (HD) in a Clevenger-type apparatus and GC-MS (HD-GC-MS) were used to extract the essential oil and analyse the volatile compounds (VOCs) respectively from *Vitex doniana* sweet fruit. 24 different volatile compounds (VOCs) were identified and grouped into eight classes of organic compounds comprising of 6 terpenes, 5 carboxylic acids, 3 ethers, 2 alcohols, 2 ketones, 2 lactones, 2 aldehydes and 2 esters. It is noteworthy that GC-MS following hydrodistillation offers invaluable information about the aroma components of the fruit, because fingerprints of volatile compounds profiles using novel extraction methods are currently highly valued due to its importance to sensory properties of foodstuffs. Therefore, characterization of the aroma compounds as markers in putrefied and fresh fruits and their products are of great importance as a quality control parameter. The sugars were then identified using a combination of 1D ^{1}H NMR and GC-MS. Characterization of the specific sugars in black plum fruit was done using GC-MS spectroscopic techniques via derivatization. This method converts the sugars in the sample to the respective trimethylsilyl-derivatives of the sugars, which are thermally stable, volatile and amenable for GC-MS analysis. The sugars identified are Alpha.-D-Glucopyranose, Glucopyranose, D-Glucose, d-(+)-Xylose, 2-Deoxy-pentose, Glucofuranoside, beta.-D-Galactopyranoside, D-Fructose, alpha.-DL-Arabinofuranoside, alpha.-DL-Lyxofuranoside, Ribitol, 2-Keto-d-gluconic acid, D-Xylofuranose and alpha.-D-Galactopyranose as obtained from their raw area percentage based on the total ion current. In conclusion, derivatization along with the coupling of GC with MS allows invaluable information about the composition and structure of sugars.

Keywords: *Aroma constituents, hydrodistillation, GC-MS, Vitex doniana, Sugar components, derivatization*

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1. Introduction

Black plum is a vegetal extensively used by numerous societies in Nigeria for countless purposes, as well as the manufacture of wine and jam. Matured black plum fruits for diet usage, usually are gathered from the ground instead of plucked [1]. *Vitex doniana* signifies some of our abandoned underutilized forestry assets. Although major research on the health benefits of plant-rich diets has laid emphasis on established vitamins, the current data are controversial and the drive towards identification of more constituents and plant food sources continues [2]. In addition, the economic importance of *Vitex doniana* is yet to be exploited to its maximum regardless of the documented uses. Blackplum of the genus verbanacae is a shrub produce that breeds in open woodland and grassland regions of tropical Africa; it is the most predominant of the Vitex species in West Africa. It yields fruits which are plum-like, sweet-smelling and fit for human consumption. It is grassland specie and hence can originate in northern, western as well as eastern Nigeria. Flowers as well as other components of natural source have being useful all over the world for human and animal health maintenance for a very long time. This is particularly in Africa where underdevelopment and dearth have made a huge proportion of the populaces depend more or less totally on traditional therapeutic practices and folkloric application of vegetation [3].

Flowers, leaves and fruits of vegetation release aroma combinations that travel through the air and are perceived by the olfactory system of animals. The intricacy of aroma is still puzzling because odor of some flower or vegetal is not owing to a particular chemical component. Even though vegetation and flowers comprise of countless chemical combinations, all of them do donate in the scent. Aroma is a combination of volatile components with a molecular weight <300 plus great vapor pressure, but the wide-ranging group of volatile components encompasses thousands of inorganic as well as organic compounds emanating from key pathways of secondary metabolism [4]. These aroma particles behave as semiochemical (blend or compound that transmits a communication for the purpose of message), pheromones, protection mechanism, and permits animals to identify and notice individuals. The pheromone aroma particles are crucial for copulating choices, sexual performance, reproduction, and nursing in addition to caution kinfolks in circumstances of hazard (alarm pheromones) and to guard against predators. Aroma particles are likewise involved in communication and collaboration like plant-plant interaction [5] and plant-animal interactions [6]. These connections and communications were achieved via cross-fertilization [7], while an additional attribute of aroma molecule is a plant's protection response against herbivores [8]. Microorganisms too have a fortune of aroma particles with an effect on fungi, animals, plants, and bacteria [9,10]. Aroma components could likewise be found in diet, spices, fragrances, wine, essential oils and fragrance oils. These components form biochemically for the duration of ripening of fruits and other crops. Aroma smell particles are of unlimited marketable importance, occasioning in countless applications of volatile aroma particles in study, food, health, cosmetic, and health industries.

Plant essential oils and extracts have been utilised for a lot of thousands of ages, specifically in food preservation, drugs, and alternative medication in addition to natural remedies [11]. The composition of essential oils comprise of a wide-ranging variety of volatile composites like mono- and sesquiterpenes, phenol-derived aromatic and also aliphatic constituents. In addition to antibacterial attributies, essential oil or their constituents have been presented to show antiviral, antimycotic, antitoxigenic, antiparasitic as well as insecticidal features, properties that are probably associated with the functions of their components in plant [12]. The make-up of essential oils in diverse plant classes is influenced by genetic make-up, culture settings and surroundings, and lastly, by crop as well as post-crop handling [13].

A diversity of procedures for extraction of essential oils from plant resources are displayed in the literature, comprising hydrodistillation [14,15], steam distillation [14,16,17], solvent extraction [14], supercritical fluid extraction [14,18,19]. Solid-phase microextraction [20], solvent free microwave microextraction (SFME) [15,21], pressurized liquid extraction [22] etc. The essential oil extract are analysed by several chromatographic procedures like high-performance liquid chromatography (HPLC) in addition to thin layer chromatography (TLC), though owing to great volatility of the analyte, the precise method is gas chromatography (GC) [23]. Gas chromatography was showcased as possibly most potent separation technique for essential oils [24]. Amongst the most commonly used procedures for quantitative estimation of essential oils are gas chromatography with mass spectroscopy (GC-MS) [25,26,27] and gas chromatography with flame ionization detector (GC-FID) [28,29]. Essential are made up of volatile aroma components and are extracted from natural botanic sources.

Derivatization is the procedure of modifying a compound chemically to yield a compound which has possessions which will be very amenable for analysis using a GC or HPLC is known as Derivatization. The most commonly used trimethylsilylation reagent for derivatization is the Tri-Sil HTP Reagent (HDMS: TMCS: Pyridine). For GC analysis and biochemical synthesis the reagent rapidly produces TMS derivatives of polar compounds. The versatile chemical reagent is ideally suited for GC determinations of a range of compounds such as phenols, steroids, sterols, alcohols, organic acids, sugars and some amines.

The aim of this study is the gas chromatography mass spectroscopy (GC-MS) determination of the volatile aroma and sugar components from *Vitex doniana* fruit. None of erstwhile research has studied the aroma compounds of *Vitex doniana* fruit using hydrodistillation gas chromatography mass spectroscopy (HDE-GC-MS).

2. Materials and Methods

2.1. Plant Collection and Identification

The collection and extraction was done using the method described by Abu [30] and Aiwonegbe, Iyasele, & Izevbuwa [31]. The fresh fruits of black plum (*Vitex doniana* sweet) (purple black in colour) (Figure 1) (1500 fruits) weighing 3 kg were collected manually by hand picking ripe fruits that fell on the ground from several randomly selected trees in a farm site in Uromi, Esan North-East Local Government Area of Edo state at the end of August, 2018 during the fruiting season (June-November, annually). The fruits were transported to the laboratory in a polypropylene (PP) box (180 x 110 x 80 mm) manufactured with holes (total area of holes 14.3 cm^2) and then stored at (ambient temperature, 85% Relative Humidity). The fruits were 2.8-3.2 cm in length, 1.2-1.4 cm in width and contained one hard conical seed each which is about 1.5-2.0 cm long and 1.0-1.2 cm wide. The plant was identified and authenticated by the Ethnobotanist in the Department of Medicinal Plant Research and Traditional Medicine (MPR&TM) of the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. A reference voucher specimen number NIPRD/01/03/CCPF/384/3 was deposited at the herbarium of the department.

Figure 1. Ripe matured *Vitex doniana* sweet fruit

2.2. Extraction of the Syrup from *Vitex doniana* Fruit

Extraction was carried out using a modified method of Aiwonegbe, Iyasele, & Izevbuwa [31]. The fruits were sorted to select the fresh ones according to similar size, firmness and colour [32] and then cleansed to remove sand and other debris, thereafter, washing with portable water and removal of the thin epicarp. The fruits were then milled through an international standard 90 µm sieve to press out the succulent mesocarp and separate the stony seed from the pericarp. The fruit pulp (1000 g) was blended with 1400 ml of distilled water in a waring blender for 10 seconds. The black honey-like syrup which weighed 1.5 kg was immediately packaged in capped bottle containers. The syrup was stored in the refrigerator at 4°C before analysis.

2.3. Fractionation of Aroma Compounds of *Vitex doniana* Sweet

Hydrodistillation (HD) and GC-MS (HD-GC-MS) were used to extract and analyse the volatile compounds (aroma) respectively from black plum fruit syrup, according to the method described by Okhale, Ugbabe, Oladosu, Ibrahim, Egharevba, Kunle, Elisha, Chibuike, & Ettah [33]. A Clevenger type apparatus under optimal operating condition was used for the purpose of hydrodistillation of essential oils from *Vitex doniana* sweet fruit syrup. A mixture of the *Vitex doniana* sweet fruit syrup (200 g) and 1000 ml of water was put into a 2000 ml of round bottomed flask [34]. The temperature was set at 80°C for the extraction of essential oil. The process in the Clevenger apparatus was run for the time till no further oil could be extracted which lasted for 3 hours. The essential oil was vaporized with the steam, condensation occurs as the vapors of the essential oil and steam mixture passed a condenser. The condensate, a mixture of oil and water, was then separated. Essential oil been lighter, settled above water and it was collected, dried over anhydrous sodium sulphate and concentrated to 1 ml [35], and stored in sealed vials in the dark at 4°C, until used. The concentrated syrup of black plum was redissolve in hexane and then used for GC-MS analyses. The experiment was carried out in triplicate.

2.4. GC-MS Analyses of *Vitex doniana* Sweet Aroma Compounds

According to the method of Okhale, Ugbabe, Oladosu, Ibrahim, Egharevba, Kunle, Elisha, Chibuike, & Ettah [33], the chemical composition of the *Vitex doniana* sweet fruit syrup essential oils extracted by hydrodistillation is performed by gas chromatography coupled with mass spectroscopy (GC/MS) using Shimadzu QP-2010 with QP-2010 Mass Selective Detector [MSD, operated in the EI mode (electron energy=70 eV) scan range of 45-700 amu, and scan rate of 3.99 scans/sec], and Shimadzu GC-MS solution data system. The Gas chromatography column was HP-5 MS fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 mL/min. The program used for Gas chromatography oven temperature was 60-160°C at a rate of 10°C/min, then held at 160°C for 2 mins, followed by 160-280°C at a rate of 15°C/min, then again held at 280^{o}C for 4 mins. The injection port temperature was 250^{o}C; while ion source temperature was 200°C; interface temperature was 250°C. 1.0 µL of diluted sample (1% v/v in hexane) was injected using autosampler and in split mode with ratio of 20:80.

Chromatographic peaks were measured using an HP ChemStation and a Shimadzu GC-MS solution data system Software was employed for data acquisition and individual constituents were identified by comparing their mass spectra with reference odorants (known compounds), literature data and the NIST Mass Special Library/database (NIST).

2.5. GC-MS and NMR Identification of the Sugars in *Vitex doniana* Fruit

Spectroscopic techniques 1D ^{1}H NMR and GC-MS were used. For the NMR analysis, 5 mg of the sample was dissolved in deuterated DMSO (DMSO-d_6) a common solvent for NMR analysis. Then the sample was analysed with 1D ^{1}H NMR at 500 MHz to obtain the spectrum showing the chemical shift, peak multiplicity and coupling constants of the prospective sweeteners (sugars) in the sample, while the characterization of the specific sugars in black plum fruit syrup was done using GC-MS spectroscopic techniques.

2.6. Derivatization Procedure

The black plum syrup was dried in a desiccator to remove water. The dried sample was treated with 5 ml of Tri-Sil HTP reagent (Thermo Scientific, Product number TS-48999, Lot number TG-267519). This method converts the sugars in the sample to the respective trimethyl derivatives of the sugars, which are volatile and amenable for GC-MS analysis. GC-MS Analysis: Modified method of Okhale, Ugbabe, Oladosu, Ibrahim, Egharevba, Kunle, Elisha, Chibuike, & Ettah [33] was used for the GC-MS analysis.

2.7. GC-MS Analyses of *Vitex doniana* Sweet sugar Components

The black plum syrup was derivatized with Tri-Sil HTP reagent and analyzed by GC-MS using Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range of 45-700 m/z, and scan rate of 3.99 scans/sec], and Shimadzu GCMS solution data system. The Gas chromatography column was HP-5 MS fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 mL/min. The program used for gas chromatography oven temperature was 60-160°C at a rate of 15°C/min, then held at 160^{o}C for 2 min, followed by 160-280°C at a rate of 10^{o}C/min, then held at 280°C for 2 min. The injection port temperature was 250°C. While ion source temperature was 200°C; interface temperature was 250°C. 1.0 µL of sample was injected using autosampler and in the split mode with ratio of 20:80.

Individual constituents were identified using NIST Mass Spectra Library (NIST). The percentage of each sugar reported as raw area percentage based on the total ion current.

2.8. Statistical Analysis

Statistical analyses were carried out with the statistical package BMDP [36], using the BMDP 2R program (stepwise multiple regression). Results were expressed as mean of triplicate analysis.

3. Results and Discussion

Based on the percentage of each constituents in the aroma and sugar profile as obtained from their raw area percentage based on the total ion current without standardization are shown in Table 1 and Table 2 respectively.

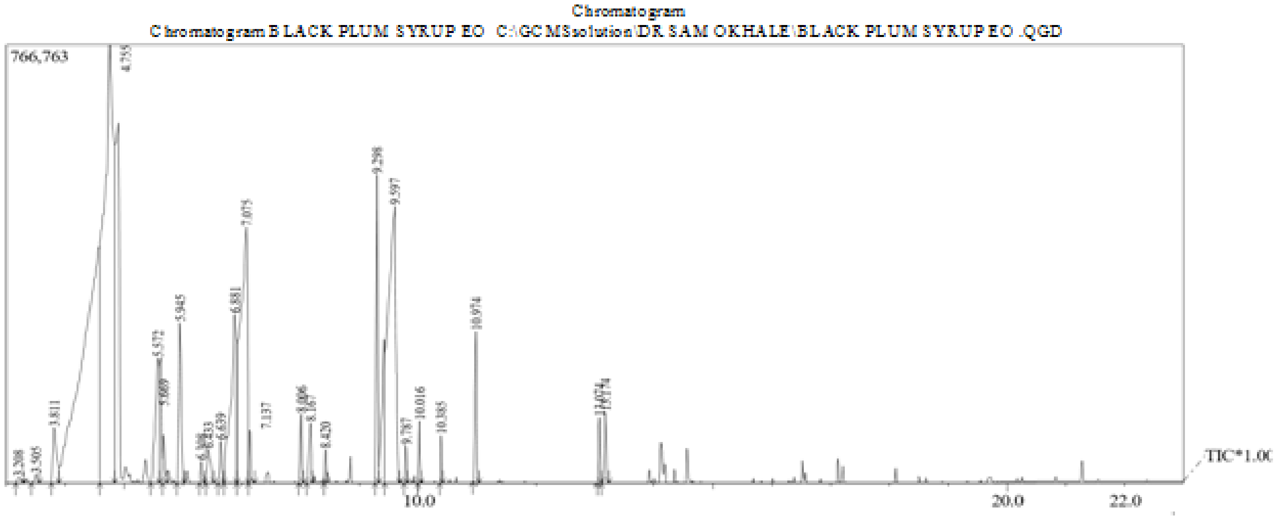

Figure 2. GC-MS Spectrum of the Aroma Components of *Vitex doniana* Fruit

Table 1. The Aroma Compounds Identified in *Vitex doniana* Fruit

Peak#	R.Time	%Composition	Name	Classification
1	3.208	0.20	Acetylfuran	Ether
2	3.505	0.22	alpha.-Thujene	Terpene
3	3.811	1.79	Benzaldehyde	Aldehyde
4	4.755	34.86	Eucalyptol	Terpene
5	5.572	3.77	Heptanoic acid	Carboxylic Acid
6	5.669	0.79	Linalool	Terpene
7	5.945	3.31	Fenchol, exo-	Terpene
8	6.308	0.35	Lilac alcohol formate C	Ester
9	6.433	0.97	Lilac aldehyde B	Aldehyde
10	6.639	0.76	p-Hydroxyacetophenone	Ketone
11	6.881	8.01	Terpinene-4-ol	Alcohol
12	7.075	14.86	n-Octanoic acid	Carboxylic Acid
13	7.137	0.61	3-Octenoic acid	Carboxylic Acid
14	8.006	0.91	4-Octanolide	Lactone
15	8.167	1.20	n-Nonanoic acid	Carboxylic Acid
16	8.420	0.43	Jasmine lactone	Lactone
17	9.298	4.29	alpha.-Terpineol acetate	Terpene
18	9.597	16.91	3-Decenoic acid	Carboxylic Acid
19	9.787	0.47	Methyl cinnamate	Ester
20	10.016	0.67	Methyleugenol	Ether
21	10.385	0.52	Caryophyllene	Terpene
22	10.974	2.24	Cycloheptanone, 3-butyl-	Ketone
23	13.074	0.83	Spathulenol	Alcohol
24	13.174	1.04	Caryophyllene oxide	Ether
		100		

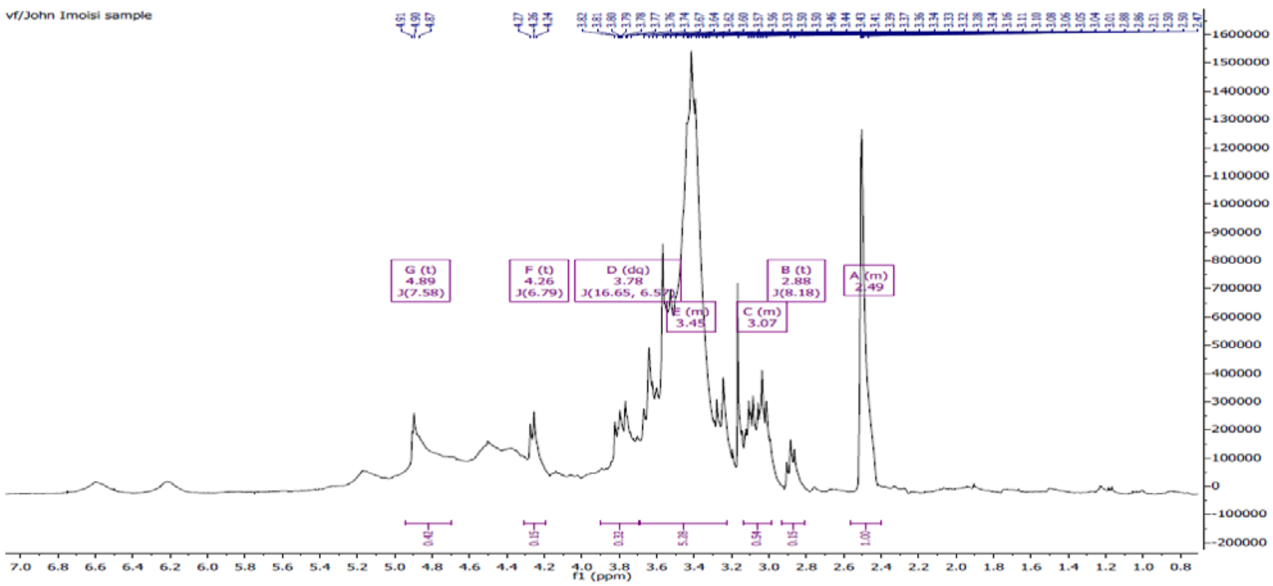

Figure 3. ^{1}H NMR Spectrum of the Sugars in *Vitex doniana* fruit

From Figure 3 above, the fruit was seen to contain a very high percentage of sugars.

Typical ^{1}H NMR chemical shifts of carbohydrate ring protons for sugars are 3-6 ppm (4.5-5.5 ppm for anomeric protons).

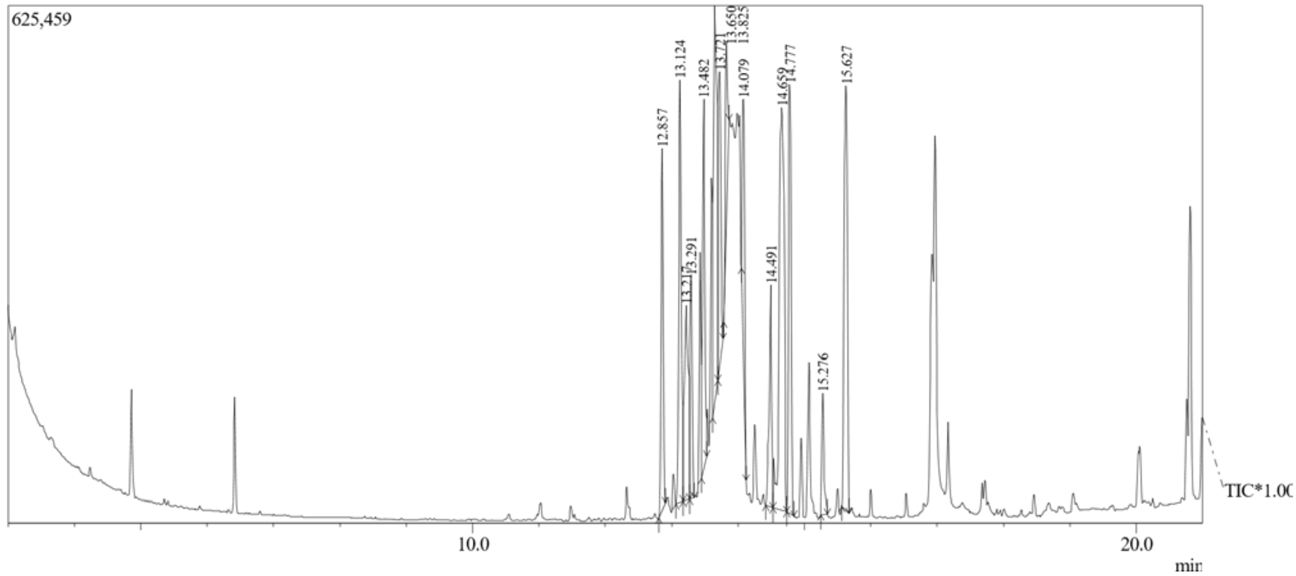

Figure 4. GC-MS Spectrum of the Sugar Components of *Vitex doniana* Fruit

Table 2. Sugars and Trimethylsilyl Sugar Derivatives of *Vitex doniana* Fruit

PEAK	Name of sugar (as the trimethylsilyl derivative)	Sugars
1.	1, 3, 4, 5, 6-pentakis-O-(trimethylsilyl)-	D-Fructose
2.	3, 4, 5-tris-O-(trimethylsilyl)-pentose	2-Deoxy-ribose
3.	Methyl 2, 3, 5- tris-O-(trimethylsilyl)-	alpha.-DL-Arabinofuranoside
4.	Pentakis-O-(trimethylsilyl)-	2-Keto-d-gluconic acid
5.	Methyl 2, 3, 5, 6-tetrakis-O-(trimethylsilyl)-	Glucofuranoside
6.	1, 2, 3, 4, 6-pentakis-O-trimethylsilyl)-	Glucopyranose
7.	Methyl 2, 3-bis-O-trimethylsilyl)-	beta.-D-Galactopyranoside
8.	1, 2, 3, 5-tetrakis-O-(trimethylsilyl)-	D-Xylofuranose
9.	Methyl 2, 3, 5-tris-O-(trimethylsilyl)-	alpha.-DL-Lyxofuranoside
10.	1, 2, 3, 4, 5-pentakis-O-(trimethylsilyl)-	Ribitol
11.	1, 2, 3, 4, 6-pentakis-O-(trimethylsilyl)-	alpha.-D-Glucopyranose
12.	Tetrakis-O-(trimethylsilyl)-ether	d-(+)-Xylose
13.	1, 2, 3, 4, 6-pentakis-O-(trimethylsilyl)-	alpha.-D-Galactopyranose
14.	2, 3, 4, 5, 6-pentakis-O-(trimethylsilyl)-	D-Glucose

Most frequently, the glycosidically-bound aroma components are released all through the industrialized handling or pretreatment of fruits. This generally induces modification to the aroma notes of those fruits [4]. Consequently, there is the need to comprehend the impact of these aroma combinations to the whole aroma of a particular fruit. Volatile aroma components consist of chemically varied class of little molecular weight organic combinations with substantial vapor pressure [4]. Nevertheless, aroma components formed by vegetation, mostly appeal to pollinators, seed dispersers as well as offer protection against pests or pathogens [8]. Nonetheless, in humans nearly 300 active olfactory receptor genes are involved to perceive thousands of diverse aroma components in addition to modulates expression of diverse metabolic genes regulating human psychophysiological activity, intelligence function, pharmacological signaling, as well as healing potential [4]. Similar to numerous stone fruits, plums (*Vitex doniana* fruit) are treasured by customer all over the ecosphereplus, subsequently, have added great commercial significance. The fruits are currently cultivated through tropical Africa and yonder [5]. The crop is both marketed fresh and utiised as dried fruit and to make juice. It is likewise utilised to manufacture jams in addition to other recipes or fermented to make wine as well as brandy. Aside from the hue, sweetness and texture, plums are particularly prevalent for their distinctive aroma [6].

In general, pure essential oils can be subdivided into two distinct groups of chemical constituents; the hydrocarbons which are made up almost exclusively of terpenes (monoterpenes, sesquiterpenes and diterpenes) and the oxygenated compounds which are mainly esters, aldehydes, ketones, alcohols, phenols, lactones, oxides and carboxylic acids.

The fruit is composed chiefly of varied classes of combinations, comprising terpenes, carboxylic acids, esters, ether, aldehyde, ketones and lactones. In all, 24 diverse volatile compounds were identified and were categorized into seven classes of organic compounds encompassing of 6 terpenes, 5 carboxylic acid, 3 ethers, 2 alcohols, 2 ketone, 2 lactones, 2 aldehydes and 2 esters.

Numerous kinds of fresh fruits were reported to yield distinctive volatile profiles. Even though an overwhelming quantity of chemical compounds have been recognized as volatile compounds in fresh fruits, simply a fraction of these compounds have been well-known as impact components of fruit aroma [11].

Terpenes were found to be the most abundant volatile constituents as they accounted for the largest proportion of the total aroma (43.99%). The terpenes are alpha-thujene (0.22%), linalool (0.79%), eucalyptol (34.86%), Fenchol-exo (3.31%), alpha terpineol acetate (4.29%) and caryophyllene (0.52%). The next most abundant compound, were carboxylic acid comprising (37.35%) of total of the volatile components identified. The carboxylic acids components are heptanoic acid (3.77%), n-octanoic acid (14.86%), 3-octenoic acid (0.61%), n-nonanoic acid (1.20%) and 3-Decenoic acid (16.91%). Many carboxylic acids are colorless liquids with disagreeable odors. The alcohols are 3-butyl spathulenol (0.83%) and terpinene-4-ol (8.01%) comprising of (8.84%) of the total volatile components identified. The next most abundant components were ketones comprising (3.00%) of the total volatile components identified. The ketones are P-hydroxylactophenone (0.76%) and cycloheptanone (2.24%). The next most abundant compound are the aldehydes comprising (2.76%) of the total volatile components identified. The aldehydes are benzaldehyde (1.79%) and lilac aldehyde B (0.97%). The ethers are acetylfuran (0.20%), methyleugenol (0.67%) and caryophyllene oxide (1.04%) comprising (1.91%) of total the volatile components identified. The lactones are 4-octanolide (0.91%) and Jasmine lactone (0.43%) comprised of (1.34%) of the total volatile components identified. The esters methyl cinnamate (0.47%) and lilac alcohol formate C (0.35%) comprised of (0.82%) representing the least total volatile components identified. Most of these compounds were formerly reported in numerous fruits like lychee, strawberry, cherry plus orange both in the free and bound state [9]. Volatile esters are made by practically all fruit classes all through ripening. Most volatile esters have aroma features termed as fruity [9,10].

The sugars identified in *Vitex doniana* fruit are fourteen (14) and are presented in Table 2, Alpha.-D-Glucopyranose is the highest with a value of (16.11%), while, the next are in the following order Glucopyranose (11.19%), D-Glucose (11.15%), d-(+)-Xylose (8.95%), 2-Deoxy-pentose (8.92%), Glucofuranoside (6.84%), beta.-D-Galactopyranoside (6.37%), D-Fructose (6.16%), alpha.-DL-Arabinofuranoside (6.14%), alpha.-DL-Lyxofuranoside (4.85%), Ribitol (4.58%), 2-Keto-d-gluconic acid (3.62%), D-Xylofuranose (3.05%) and alpha.-D-Galactopyranose (2.07%).

4. Conclusion

It is noteworthy that GC-MS following hydrodistillation offers invaluable information about the aroma components of the fruit, because fingerprints of volatile compounds profiles using novel extraction methods are currently highly valued due to its importance to sensory properties of foodstuffs. Therefore, characterization of the aroma compounds as markers in fresh fruits and their products are of great importance as a quality control parameter. Gas chromatography is a technique used to separate and quantitate components in a mixture. GC Coupled with mass spectrometry is a powerful technique that provides both qualitative verification of the identity of a chromatographic peak and quantitation. *Vitex doniana* fruit has a unique flavor due to the presence of 24 aroma components with terpenes and esters being the highest and least respectively in terms of the quantity and numbers of identified compounds. The study has shown that the edible pulp of black plum (*Vitex doniana*) fruit is a good source of sugars, and that acceptable syrup could be solely produced from the extracted juice. The syrup is an intermediate moisture food with very high sugar content and fourteen (14) different types of sugars account for its unique taste.

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