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Determination of Aroma Components in *Vitex* doniana Fruit Syrup Following Hydro distillation Extraction

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Abstract

The fresh fruits of black plum (Vitex doniana) were collected from several randomly selected trees in a farm site in Uromi metropolis, Esan North-East Local Government Area of Edo state and then processed into an extract in form of syrup. Hydro distillation extraction (HDE) and GCMS (HDE-GC-MS) were used to extract and analyse the volatile compounds, VOCs (aroma) respectively from black plum (Vitex doniana) fruit syrup and the percentage of each components were reported as raw area percentage based on the total ion current. 24 different volatile compounds (VOCs) were identified and were grouped into seven classes of organic compounds comprising of 8 terpenes, 5 carboxylic acids, 4 ketones, 2 lactones, 2 aldehydes, 2 ethers and 1 ester. The terpenes are alpha-thujene (0.22%), eucalyptol (34.86%), linalool (0.79%), Fenchol-exo (3.31%), terpinene-4-ol(8.01%), alpha terpineol acetate (4.29%), 3-butyl spathulenol (0.83%) and caryophyllene (0.52%). 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%). The ketones are acetylfuran (0.20%), lilac alcohol format C (0.35%), P-hydroxylactophenone (0.76%), and cycloheptanone (2.24%). The aldehydes are benzaldehyde (1.79%) and lilac aldehyde B (0.79%). The Ethers are methyl eugenol (0.67%) and caryophyllene oxide (1.04%). The lactones are 4-octanolide (0.91%) and Jasmine lactone (0.43%). Esters represent the least of the total volatile compounds identified, which is methyl cinnamate (0.47%). The aroma components are the characteristic odour active compounds in Vitex doniana fruit syrup. The aroma components could also contribute to the biomedical activities of the syrup, especially its antioxidant effect due to their natural radical scavenging potential.

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Introduction

Black plum a plant largely needed by several households in Nigeria for many applications, including production of jam and wine. Matured black plum fruits for food use, usually are not plucked instead they are picked from the ground (Okigbo, 2001). There is the need for better understanding of available plants including the severally underutilized species with increasing sensitization on improving plant food resources in Nigeria. *Vitex doniana* represents one of our underutilized neglected traditional plant resources. Although major work on the health benefits of plant-rich diets have lied emphasis on established vitamins, the current data are controversial and the drive towards identification of more constituents and plant food sources continues (Ochieng and Nandwa, 2010). In addition, the economic relevance of *Vitex doniana* has not been fully exploited to its maximum inspite the documented uses. Blackplum of the genus verbanacae is a tree crop that grows in savannah and open woodland regions of tropical Africa; in West Africa it is the commonest of Vitex species. It produces fruits which are edible, sweet and plum like. The fruit is changes to dark brown when fully ripe but green when mature, containing 1 to 4 seeds is the pulp surrounding a hard stone. It is a savannah specie and therefore can be found in western, eastern and northern Nigeria. For agelong time throughout the world for animal and human health care, plants resource of natural origin have being applied. This is especially in Africa where poverty and underdevelopment have made a large percentage of the people depend almost totally on folkloric application of plants and traditional medical practices (Enzo, 2006). The potency has been shown by several researchers of some of these traditional herbal

remedies. One of those plants popular for it wide use in Africa native folklore is *V. Doniana*. The plant is indigene to Botswana, Nigeria, Kenya, Ethiopia, Namibia, Lesotho, Senegal, Niger, South Africa, Somalia, Tanzania, Sudan, Zambia and Uganda. It is locally known as oori-nla (Yoruba), dinya (Hausa), Vitex (English) and ucha koro (Igbo) (Burkill, 2000). Various parts of the plant are used as remedy for infectious conditions in traditional medicine, such as anaemia, infertility, leprosy, jaundice, colic, dysentery, backaches, gonorrhoea, febrifuge, headaches, conjunctivitis and other eye troubles, measles, stiffness, fever, rash, hemiplegia, chickenpox, as tonic galactagogue to aid milk production in lactating mothers, dearth of vitamin A and B, ankylostomiasis (ancylostomiasis), anodyne, leprosy and liver disease, rachitic and kidney troubles. For cleaning the teeth, the twigs are used as chewing sticks. The blackish extract gotten by boiling the bark, leaves, fruits and/or root is applied as dye and ink for clothes (Burkill, 2000). 'Vitex', the generic name, is a Latin ancient name for the genus. Aside from the commercial importance of this plant in wood and timber production, not much research has been done on its antimicrobial activity and chemical. It is in the light of the above, in drug discovery, that this work aim to link scientific findings with some of these folkloric uses with the intentions of bringing more research interest to plant in trending hit/lead prospects. Black plum are branded as juicy mesocarp seed bearing structure of flowering plant that may be consumed as food (Hyson, 2002).

Materials and Methods

Fresh fruits of *Vitex doniana* (black plum) were collected from several randomly selected trees in a farm site in Uromi metropolis, Edo state, Esan-North East Local Government Area. The plant was identified and ascertained by the Ethnobotanist and registered with a voucher specimen number NIPRD/01/03/CCPF/384/3 and deposited at the herbarium of (NIPRD), Idu Industrial area, Abuja, that is the National institute for pharmaceutical research and development. Extraction was carried out using a modified method of Aiwonegbe (2018). The fruits were kept under ambient temperature in the laboratory. The fruits were sorted to select the fresh ones and then cleansed to remove sand and other debris. Thereafter, portable water was used for washing the fruit pulp and the thin epicarp was removed. The fruits were then milled through a 90 µm sieve to press out the succulent mesocarp and separate the stony seed from the pericarp. In a waring blender for a few seconds, the fruit pulp was blended and warm water at 30°C was added to the mixture. For five minutes, the mixture was then stirred continuously with a wooden paddle to obtain the syrup.Hydro distillation extraction (HDE) and GC-MS (HDE-GC-MS) were used to extract and analyse the volatile compounds (aroma) respectively from black plum (*Vitex doniana*) fruit syrup. According to the method described by Okhale et al. (2018). Volatile constituents of the sample were obtained by hydro distillation and analysed by 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^{0}C at a rate of 10 ^{0}C/min, then held at 160^{0}C for 2 min, followed by 160-280^{0}C at a rate of 15^{0}C/min, then again held at 280^{0}C for 4 min. The injection port temperature was 250^{0}C while ion source temperature was 200^{0}C; interface temperature was 250^{0}C. 1.0 µL of diluted sample (1% v/v in hexane) was injected using auto sampler and in split mode with ratio of 20:80. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Special Library (NIST). The percentage of each component was reported as raw area percentage based on the total ion current.

Discussion

Based on the percentage of each constituents and class of organic compounds contribution to the aroma profile as obtained from their raw area percentage based on the total ion current without standardization from table 1. Like many stone fruits, plums (*Vitex doniana* fruit) are appreciated by consumer all over the world and, consequently, have gained great economic importance. The fruits are presently cultivated across tropical Africa and beyond. The crop is either marketed fresh or used as dried fruit and to produce juice. It is also used to make jams and other recipes or fermented to produce wine and brandy. Apart from colour, sweetness and texture, plums are especially popular for their characteristic aroma. The produced syrup is comprised mainly of diverse classes of compounds, including terpenes, carboxylic acids, esters, ether, aldehyde, ketones and lactones. In all, 24 different volatile compounds were identified and were grouped into seven classes of organic compounds comprising of 8 terpenes, 5 carboxylic acid, 4 ketone, 2 lactones, 2 aldehydes 2 ethers and 1 ester. Various types of fresh fruits were reported to produces distinct volatile profiles. Although an overwhelming number of chemical compounds have been identified as volatile compounds in fresh fruits, only a fraction of these compounds have been identified as impact compounds of fruit flavor based on their quantitative abundance and olfactory threshold. Terpenes were found to be the most abundant volatile constituents as they accounted for the largest proportion of the total aroma (33.3%) and also had the highest impact of (52.83%) on the aroma note or odour activity of the syrup. The terpenes are alpha-thujene: 0.22%, eucalyptol: 34.86%, linalool: 0.79%, Fenchol-exo: 3.31%, terpinene-4-ol: 8.01%, alpha-terpineol: 4.29%, 3-butyl spathulenol: 0.83% and caryophyllene: 0.52%. Terpene are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, and by some insects (Davis and Croteau, 2000). They often have a strong odour and may protect the plants that produce them by deterring herbivores and by attracting predators and parasites of herbivores (Martin et al., 2003). Although sometimes used interchangeably with "terpenes", tepenoids (or isoprenoids) are modified terpenes as they contain additional functional groups, usually oxygen-containing (Martin et al., 2003). Terpenes are hydrocarbons. Terpenes are the major components of rosin and turpentine produced from resin. The name "terpene" is derived from the word "turpentine". Terpenes are also major biosynthetic building blocks. Steroids, for example, are derivatives of the triterpene

Table 1: The Aroma Compounds Identified in Vitex donianaFruit Syrup.

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.208	3.158	3.300	49985	0.20	9714	0.21	5.15		Acetylfuran
2	3.505	3.425	3.567	57036	0.22	16211	0.36	3.52		alpha.-Thujene
3	3.811	3.758	3.892	453424	1.79	97395	2.14	4.66		Benzaldehyde
4	4.755	4.583	4.842	8849198	34.86	766763	16.82	11.54	V	Eucalyptol
5	5.572	5.458	5.592	956952	3.77	218475	4.79	4.38		Heptanoic acid
6	5.669	5.650	5.725	201372	0.79	85019	1.86	2.37	V	Linalool
7	5.945	5.892	6.025	840574	3.31	281068	6.16	2.99		Fenchol, exo-
	6.308	6.275	6.367	88737	0.35	38549	0.85	2.30		Lilac alcohol formate C
8	6.433	6.383	6.525	246579	0.97	59119	1.30	4.17		Lilac aldehyde B
9	6.639	6.600	6.692	191765	0.76	74108	1.63	2.59		p-Hydroxyacetophenone
10	6.881	6.692	6.917	2033091	8.01	295672	6.48	6.88	V	Terpinene-4-ol
11	7.075	6.917	7.117	3773673	14.86	448270	9.83	8.42	V	n-Octanoic acid
12	7.137	7.117	7.217	154047	0.61	92621	2.03	1.66	V	3-Octenoic acid
13	8.006	7.967	8.050	229973	0.91	121437	2.66	1.89		4-Octanolide
14	8.167	8.100	8.200	304582	1.20	106339	2.33	2.86		n-Nonanoic acid
15	8.420	8.392	8.442	108374	0.43	58919	1.29	1.84		Jasmine lactone
16	9.298	9.258	9.333	1090228	4.29	540188	11.85	2.02		alpha.-Terpineol acetate
17	9.597	9.433	9.675	4293362	16.91	485156	10.64	8.85	V	3-Decenoic acid,
18	9.787	9.758	9.817	118835	0.47	65850	1.44	1.80		Methyl cinnamate
19	10.016	9.983	10.042	169359	0.67	109387	2.40	1.55		Methyleugenol
20	10.385	10.358	10.417	133058	0.52	83965	1.84	1.58		Caryophyllene
21	10.974	10.925	11.033	568259	2.24	264640	5.80	2.15		Cycloheptanone, 3-butyl-
22	13.074	13.042	13.108	210918	0.83	116288	2.55	1.81		Spathulenol
23	13.174	13.108	13.242	264465	1.04	124756	2.74	2.12	V	Caryophyllene oxide

squalene. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers (Davis and Croteau, 2000). Essential oils are used widely as fragrances in perfumery and traditional medicine, such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavour's used in food additives. Thujene which is commonly known as alpha thujene (3-thujene) and less commercially as 2-thujene, the IUPAC name 1-Isopropyl-4-methylbicyclo [3.1.0] hex-3-ene is a natural organic compound classified as a monoterpene. It is found in the essential oils of a variety of plants, and contributes pungency to the flavor of some herbs such as summer savoury, *Vitex doniana* fruit. Eucalyptol is a natural organic compound that is a colourless liquid. It is a cyclic ether and a monoterpenoid. Eucalyptol comprises up to 90% of the essential oil of some species of the generic product eucalyptus oil (Boland et al., 1991), hence the common name of the compound. It is also found in camphor laurel, bay leaves, tea tree, sweet basil, wormwood, rosemary, common sage, cannabis sativa and other aromatic plant foliage. Eucalyptol with a purity from 99.6 to 99.8% can be obtained in large quantities by fractional distillation of eucalyptol oil. Although it can be used internally as a flavouring and medicine ingredient at very low doses, typically of many essential oils (volatile oils), eucalyptol is toxic if ingested at higher than normal doses. Because of its pleasant, spicy aroma and taste, eucalyptol is used in flavourings, fragrances, and cosmetics. Eucalyptol has a fresh mint-like smell and a spicy, cooling taste. Terpinen-4-ol is an isomer of terpineol with the chemical formula $C_{10}H_{18}O$. A primary constituent of tea tree oil, it is obtained as an extract from the leaves, branches, and bark of Melaleuca alternifolia Cheel (Hammer et al., 2012). It may be a factor in the contact dermatitis of tea tree oil when used topically (Hammer et al., 2012). Linalool refers to two enantiomers of a naturally occurring terpene alcohol found in many flowers and spice plants. Found in many flowers and spice plants. These have multiple commercial applications, the majority of which are based on its pleasant scent (floral, with a touch of spiciness). It can be called linalyl alcohol. Alpha Terpineol is a monoterpene alcohol that has been isolated from a variety of sources such as cajuput oil, pine oil, and petit grain oil. There are four isomers, alpha-, beta-, gammaterpineol, and terpinen-4-ol. Beta and gamma-terpineol differ only by the location of the double bond. It has a pleasant odour similar to lilac and is a common ingredient in perfumes, cosmetics, and flavour's. It is one of the two most

abundant aroma constituents of lapsang souchong tea. It originates in the pine smoke used to dry the tea (Yuasa, 2006). 3-butyl spathulenol is sesquiterpene alcohol first isolated from the essential oils of *Artemisia vulgaris* and *Artemisia dracunculus*. It has an earthy herbal fruity aroma, 3-butyl spathulenol also shows the capacity to inhibit proliferation in the lymphocytes, inducing apoptosis in these cells possibly through a caspase-3 independent pathway. Immunoinhibitory effect. Caryophyllene, is a natural bicyclic sesquiterpene that is a constituent of many essential oils, especially clove oil, the oil from the stems and flowers of *Syzygiumaromaticum* (cloves) (Ghelardini *et al*., 2001), the essential oil of cannabis sativa, rosemary, and hops (Ormeno *et al*., 2008). It is found as a mixture with isocaryophyllene (the *cis*-double bond isomer). Caryophyllene is notable for having a cyclobutane ring, as well as a trans-double bond in a 9membered ring. The next most abundant compound, were carboxylic acid comprising (20.8%) of the total volatile components identified and had an impact of (37.35%) on the aroma note or odour activity of the syrup. Many carboxylic acids are colourless liquids with disagreeable odours. The carboxylic acids with 5 to 10 carbon atoms all have "goaty" doors (explaining the odour of Limburger cheese). Unlike esters which have sweet pleasant odours often associated with fruits, carboxylic acids are noted for their sour, disagreeable odours. The carboxylic acids components are heptanoic acid: 3.77%, noctanoic acid: 14.86%, 3-octenoic acid: 0.61%, n-nonanoic acid: 1.20% and 3-Decenoic acid: 16.91%. The molecular weight of organic acids varies widely from relatively small compounds (fatty acids) with higher numbers of carboxylic and phenolic functional groups. Monocarboxylic acids with 5-10 carbon with unpleasant smells. As the carbon chain length increases (greater than 10 carbon atoms) the acids are warlike solids, and their smell diminishes with increasing molar mass and decreasing volatility. The next most abundant components were ketones comprising (16.7%) of the total volatile components identified and had impact of (3.55%) on the aroma note or odour activity of the syrup. The ketones are acetylfuran:0.20%, lilac alcohol format C: 0.35%, P-hydroxylactophenone: 0.76%, and cycloheptanone: 2.24%. Ketones impart some very characteristics fragrance in compounds. For instance, ketones help in the formation of a compound "acetophenone" which is responsible for fragrances such as cherry, jasmine, honeysuckle, almond, strawberry, etc. Acetyfuran is a sweet balsam almond, nutty, brown and toasted with a milky, lactonic undernote. It is used in Chocolate, Coffee, Roast nut, Bread, Rum, Whiskey, Tamarind, Tea and Tobacco flavours, as a trace background note. 2-Acetylfuran is the most abundant flavor compound in tamarind, where its aroma, in conjunction with alpha-terpineol, citral and some trace pyrazines, contributes to the flavor. Cyclohexanone is the organic compound with the formula $(CH_2)_5CO$. The molecule consists of a six carbon cyclic molecule with a ketone functional group (Michael, 2005). This colourless oil has an odour reminiscent of that of acetone. Over time, samples of cycloheptanone assume a yellow colour. Cyclohexanone is slightly soluble in water and miscible with common organic solvents (Michael, 2005). P-Hydroxyacetophenone or Piceol is a phenolic compound found in the needles and in mycorrhizal roots of Norway spruces (*Picea abies*) (Lokke, 1990). P-Hydroxyacetphenone monooxygenase is an enzyme that transforms piceol into 4-hydroxyphenylacetic acid. This enzyme is found in *Pseudomonas fluorescens* (Lokke, 1990). The next most abundant compound, were aldehyde (benzaldehyde: 1.79% and lilac aldehyde B: 0.79%) comprised of (8.33%) and had impact of (2.76%) on the aroma note or odour activity of the syrup. Benzaldehyde is a characteristic constituent of skin waxes of plums having a fragrant, woody-like aroma (Williams and Ismail, 1981). Benzaldehyde is an organic compound consisting of a benzene ring with a formyl substituent. It is the simplest aromatic aldehyde and one of the most industrially useful. It is a colourless liquid with a characteristic almond-like odour. The primary component of bitter almond oil, benzaldehyde can be extracted from a number of other natural sources (Scott and Scott, 1920). Synthetic benzaldehde is the flavouring agent in imitation almond extract, which is used to flavor cakes and other baked goods (Adams et al., 2005). Ethers (methyl eugenol: 0.67% and caryophyllene oxide: 1.04%) comprising (8.33%) of total the volatile components identified and had impact of (1.71%) on the aroma note or odour activity of the syrup. Methyl eugenol is a natural chemical compound classified as a phenyl propene, a type of phenylpropanoid. It is the methyl ether of eugenol and its important behaviour and pollination (Tan and Nishida, 2012). It is found in various essential oils. Methyl eugenol is found in a number of plants (over 450 species from 80 families including both angiosperm and gymnosperm families) and has a role in attracting pollinators. About 350 plant species have them as a component of floral fragrance. The compound (methyl eugenol) can be evolved in response to pathogens, as it has some antifungal activity. It also repels many insects (Tan and Nishida, 2012). Caryophyllene oxide is the oxidized form of caryophyllene, one of the terpenes found in cannabis, as well as one of the major terpenes found in cloves, basil, hops, pepper, and rosemary. The lactones (4-octanolide: 0.91% and Jasmine lactone: 0.43%) comprised of (8.33%) of the total volatile components identified and had impact of (1.34%) on the aroma note or odour activity of the syrup. Naturally occurring lactones are mainly saturated and unsaturated gamma and sigma lactones. They are intramolecular esters of the corresponding hydroxyl fatty acids. They contribute to the aroma of fruits, butter, cheese, and other foods (Karl-Georg et al., 2007). Cyclopentadecanolide is responsible for the masklike odour of angelica root oil. Of the naturally occurring bicyclic lactones, phthalides are responsible for the odours of celery and lovage oils, and coumarin for woodruff (KarlGeorg et al., 2007). γ -octalactones, and γ-decalactones were also considered as important lactone contributors to the aroma of fresh plums (Williams and Ismail, 1981). Jasmine lactone is a lactone and aroma compound with a powerful fatty-fruity peach and apricot flavor. Its chemical formula is $C_{10}H_{16}O_2$. It occurs naturally in jasmine oil, tuberose, gardenia, mimosa, honeysuckle, lily, tea, peach, and ginger. It is used as a food spice and is mainly used for the preparation of apricot, peach, dairy products, and as a tropical fruit flavor. 4-Octanolide is commonly known as whisky lactone or quercus lactone is an important ingredient in the aromas of whiskey and other alcoholic beverages that have been aged in oak barrels. It is sometimes added to the liquor as a flavouring agents. It has a coconut, celery or fresh wood aroma that can be detected by humans at the concentration of 1 ug/L in air (Eric et al., 2000). Esters represent the least (4.2%) of the total volatile

compounds identified which is methyl cinnamate: 0.47% and had the least impact of (0.47%) on the aroma note or odour activity of the syrup. Apart from methyl cinnamate, other esters are also well known in fruits for their sweet smelling fragrance, some of these esters are octyl ethanaote in orange, ethyl butanone in cherry, butyl ethanaote in banana, ethyl ethanoate in rum, linalyl butyrate in peach, linalyl acetate in lavender/sage, isobutyl acetate in strawberry, isoamyl formate in raspberry, methyl acetate in glue, methyl butyrate pineapple, methyl phenylacetate in honey, pentyl pentanoate in apple, propyl hexanoate in cheese, methyl pentanoate in flowery, methyl salicylate in wintergreen, ethyl acetate in butter, ethyl benzoate in sweet, methyl butanaote in apple, benzyl acetate in jasmine etc. Esters formed from simple hydrocarbons groups are colourless, volatile liquids with pleasant aromas and create the fragrances and flavours of many flowers and fruits. They are also used as food flavourings. Larger esters, formed from long-chain carboxylic acids, commonly occur as animal and vegetable fats, oils, and waxes. Methyl cinnamate is the methyl ester of cinnamic acid and is a white or transparent solid with a strong, aromatic odour (Boland et al., 1991). It is found naturally in a variety of plants, including in fruits, like strawberry, and some culinary spices, such as Sichuan pepper and some varieties of basil (Vina et al., 2003). Eucalyptus olida has the highest known concentrations of methyl cinnamate (98%) with a 2-6% fresh weight yield in the leaf and twigs (Boland et al., 1991). But studies of the volatile compositions of plums found that the esters are qualitatively and quantitatively the most important class compounds (Crouzet, 1990).

Conclusion

Aromas represent an extremely heterogeneous group of molecules because of their botanical origin, their functional properties, and especially for the chemical structure and the reactivity they exhibit. They are present in some food products, as natural or added parts of a more complex formulation, aroma is responsible for the singular properties of many foods. Consequently, especially when having to design new food products, it is fundamental to take into account a detailed description of the structural characteristics of its aroma components. *Vitex doniana* fruit syrup has a unique flavor due to the presence of 24 aroma components.

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