Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2020, 12(8): 01-06

Research Article

ISSN: 0975-7384
CODEN(USA): JCPRC5

Spectroscopic Determination of Sugar Components of *Vitex doniana* Fruit Syrup

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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. The sugars were identified using a combination of 1D ^{1}H NMR and GC-MS. 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 shifts, peak multiplicity and coupling constants of the prospective sweeteners (sugars) in the sample. While characterization of the specific sugars in black plum fruit syrup 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 volatile and amenable for GC-MS analysis. The sugars identified in Vitex doniana fruit syrup are fourteen and are presented based on the percentage of each sugar constituents and contribution to the sweetness profile of the syrup as obtained from their raw area percentage based on the total ion current. The sugars identified are Alpha.-D-Glucopyranose (16.11%), 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%). While the least contributor is the alpha-D-Galactopyranose (2.07%).

Keywords: Sugar components; Derivatization; *Vitex doniana*; Syrup; Spectroscopic

INTRODUCTION

Black plum (*Vitex doniana*) is a plant widely used by several communities in Nigeria for many purposes, including production of wine and jam. Ripe mature black plum fruits for food use, usually are collected from the ground instead of plucked [1]. With increasing emphasis on upgrading traditional plant food resources in Nigeria, there is the need for better understanding of available plants including the severally underutilized species. *Vitex doniana* represents one of our neglected underutilized forest resources. Although major research on the health benefits of plant-rich diets has lied 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 value of *Vitex doniana* has not been exploited to its maximum despite the documented uses. Black plum of the family verbanacae is a tree crop that grows in open woodland and savannah regions of tropical Africa; it is the commonest of *Vitex* species in West Africa. It produces fruits which are plum like, sweet and edible. The fruit is green when mature and changes to dark brown when fully ripe, with the pulp surrounding a hard stone containing 1 to 4 seeds. It is a savannah specie and therefore can be found in northern, western, and eastern Nigeria.

For age long time throughout the world for animal and human health care, plants resource of natural origin has 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 [3,4]. 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) [5,6]. Various parts of the plant are used as remedy for infectious conditions in traditional medicine, such as anaemia, infertility, leprosy, jaundice, colic, dysentery, backaches, gonorrhea, 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, rachitis 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 [6].

Derivatization is a process of chemically modifying a compound to produce a compound which has properties that are suitable for analysis using a GC or HPLC. Derivatization is a technique in chemistry which transforms a chemical compound into a product (reaction's derivate) of similar chemical structure called a derivative. The most used trimethylsilylation reagent for derivatization is the Tri-Sil HTP Reagent (HDMS: TMCS: Pyridine). The reagent rapidly produces TMS derivatives of polar compounds for GC analysis and biochemical synthesis. The versatile reagent is ideally suited for GC determinations of a range of compounds such as sugars, alcohols, phenols, steroids, sterols, organic acids, and some amines. GC and GC-MS are excellent techniques for the analysis of carbohydrates; nevertheless, the preparation of adequate derivatives is necessary. The different functional groups that can be found and the diversity of samples require specific methods. The sugar structures of black plum are unavailable though widely eaten by natives. Bello et al. [7] reported that the syrup contains 70% carbohydrates and the total sugar content accounts for over 95%. But did not specify the chemical structure of the individual sugars. Abu [8] even went on to quantify the amount of sugar present in the syrup from chemical composition evaluation but did not also specify the structure of the sugars responsible for the sweetness. This research will therefore, identify the specific chemical structure of these sugars.

MATERIALS AND METHODS

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. The plant was identified by the

Ethnobotanist and registered with a voucher specimen number NIPRD/01/03/CCPF/384/3 and deposited at the herbarium of the National institute for pharmaceutical research and development (NIPRD), Idu Industrial area, Abuja.

Extraction was done using a modified method described by Aiwonegbe et al. [9]. 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, washing with portable water and removal of the thin epicarp. The fruits were then milled through a 90 µm sieve to press out the succulent mesocarp and separate the stony seed from the pericarp. The pulp was blended in a waring blender for a few seconds and warm water at 30°C was added to the mixture. The mixture was then stirred continuously for five minutes with a wooden paddle to obtain the syrup.

Spectroscopic techniques 1D ^1H 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 ^1H 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. 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 et al. [10] was used for the GC-MS analysis.

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 150C/min, then held at 160°C for 2 min, followed by 160-280°C at a rate of 10°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.

RESULTS

From Figure 1 below, the syrup was seen to contain a very high percentage of sugars. Typical ^1H NMR chemical shifts of carbohydrate ring protons for sugars are 3-6 ppm (4.5-5.5 ppm for anomeric protons). Modern high field NMR instruments used for carbohydrate samples, typically 500 MHz or higher, can run a suite of 1D, 2D and 3D experiments to determine a structure of carbohydrate compounds. In the case of simple mono- and oligosaccharide molecules, all proton signals are typically separated from one another (usually at 500 MHz or better NMR instruments) and can be assigned using 1D ^1HNMR spectrum only.

GC-MS Results of the Chemical Structures of Sugars Components *Vitex doniana* Fruit Syrup

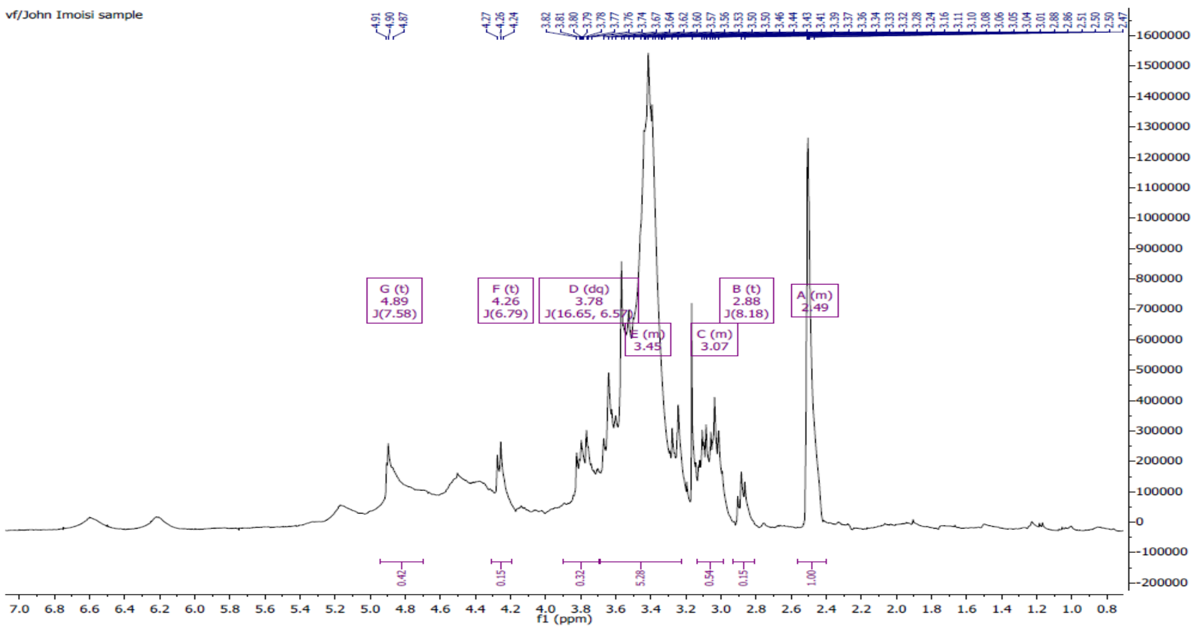

Figure 1. 1H NMR Spectrum of the Sugars in *Vitex doniana* fruit Syrup.

Table 1. Sugars and Trimethylsilyl Sugar Derivatives of *Vitex doniana* Fruit Syrup

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	Methyl 2, 3, 5- tris-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

DISCUSSION

The sugars identified in *Vitex doniana* fruit syrup are fourteen (14) and are presented in Table 1. Based on the percentage of each sugar constituents and contribution to the sweetness profile of the syrup as obtained from their raw area percentage based on the total ion current without standardization from Table 1. Alpha-D-Glucopyranose is highest with a value of (16.11%) and therefore, the largest sweetness contributor. The next highest contributor to the sweetness profile is Glucopyranose with a value of (11.19%). While the next are in the following order 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%). While the least contributor is the alpha-D-Galactopyranose (2.07%).

Carbohydrates are especially prominent constituents of plants and usually form over one-half of the total plant substance. They serve not only as a source of available energy but also as reserve food and as structural materials. They are one of the main groups of food substances (carbohydrates, proteins, and fats) to be synthesized in the plant from simple organic substances. The empirical composition of carbohydrates may be expressed by the formula CnH2nOn. About their specific chemical properties, carbohydrates may contain a potential aldehyde, -CHO, or ketone, C=O, group. In general, the substances belonging to this class of compounds may be divided into three broad groups: monosaccharides, oligosaccharides, and polysaccharides. Monosaccharides have five carbon atoms (pentoses) or six carbon atoms (hexoses) and have a sweet taste [11]. The second group of carbohydrates, oligosaccharides, is made up of two or more monosaccharide units linked to one another through a glycosidic bond. These are the disaccharides, trisaccharides, tetrasaccharides, etc., and may or may not have reducing properties. No sharp line of distinction can be drawn between the oligosaccharides and the third group of carbohydrates, the polysaccharides, which represent large aggregates of monosaccharide units (starch, cellulose, pectin, etc.). The main function of carbohydrates upon ingestion by an animal organism is that of a fuel. They are metabolized to other products with the release of carbon dioxide, water, and energy. In addition, certain products of carbohydrate metabolism aid in the breakdown of many food stuffs, acting as catalysts in biological oxidations. Carbohydrates can also be used as a starting material for the biological synthesis of other types of compounds in the body, such as fatty acids and certain amino acids. Regardless of the form in which a carbohydrate happens to be ingested, it must be transformed into a monosaccharide for absorption and metabolism, thus emphasizing the significance of monosaccharides in food stuffs. Despite the increasing awareness of the specific carbohydrate role as human food, however, many problems in this field are far from being solved owing to chemical and structural complexity of the sugars [12].

Much work has been devoted to the preparation of derivatives for different families of carbohydrates before their GC-MS analysis. These have been focused on two main aims: (i) to simplify the derivatization process; (ii) to reduce the number of peaks in the chromatogram. At present, there are several derivatization methods with one or two steps which can be satisfactorily carried out in the laboratory [13]. The large number of chromatographic peaks per sugar is still mentioned as a problem and different methods have been developed to reduce this effect. However, in certain cases multiple peaks may be an aid for carbohydrate identification. In the last few years, the number of emerging derivatization methods has been limited. These new applications are resolved with optimization and experiment designs to find specific conditions for each sample which afford the best yield and reproducibility. In certain cases, the use of two different derivatives provides important structural information, either through MS fragmentation or through retention data [13]. In conclusion, derivatization along with the coupling of GC with MS allows invaluable information about composition and structure of real samples. Silylation is used to enhance GC performance. The silyl reagents have two desirable results: increase analyte volatility and decrease surface adsorption.

Silyl derivatives are formed by displacement of active hydrogen on -OH, -SH, and -NH groups. Compounds containing active hydrogen atoms amendable to silylation are acids, alcohols, thiols, amines, amides, and enolizable ketones and aldehydes [13]. Their silyl derivatives generally are more volatile, less polar, and thermally more stable. The choice of a silyl reagent is based on its reactivity and selectivity toward the compound, the intended application, the stability of the derivative, and the abundance and nature of reaction byproducts. Sterically crowded reagents with bulkier R groups are generally less reactive but give more stable derivatives after silylation. "Silyl derivatives generally are more volatile, less polar, and thermally more stable."

CONCLUSION

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 a very high sugar content and fourteen different types of sugars account for its unique taste. The high sugar content of *Vitex doniana* syrup will probably discourage microbial growth and hence deterioration. Proper exploitation of the fruit and utilization of the syrup can help conserve foreign exchange expended on the importation of syrup, and as substitute for other syrups in industrial and food uses. It can be concluded from the characteristics of the *Vitex doniana* fruit syrup, that it is a potential source of food grade sweetener.

ACKNOWLEDGEMENT

I am indeed very grateful to the staffs and management of the Department of Chemistry, University of Benin (UNIBEN) and also those of the National Institute of Pharmaceutical Research and Development (NIPRD) for their support, and to my Supervisor, Professor J.U. Iyasele for his guidance and supervision.

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