BIOACTIVE COMPOUNDS IN HEALTH AND DISEASE COMPOSED IN SYRUP PREPARED FROM VITEX DONIANA FRUIT

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Abstract. Plant-based foods' health-promoting and disease-curing properties can be found in their rich chemicals. Evidence revealing this is the number of chemicals isolated from the plants that have been successfully employed to cure and prevent human and animal diseases. This study aimed to evaluate the chemical compounds in syrup prepared from *Vitex doniana* fruit using conventional and GC-MS methods. The phytochemicals qualitatively screened are phenols, flavonoids, alkaloids, saponins, terpenoids, and cardiac glycosides. Their quantity values are 17.437, 0.289, 19.088, 48.273, 3.139, and 26.817, respectively. Nineteen compounds were identified with the GC-MS chromatogram at a retention time that ranged from 4.727 to 24.701 min. These chemicals were confirmed based on their area%, height%, structure formula, retention time, and molecular weight. The chemical compounds are glycerol, L-Mannomethylose, Ethyl β-d-riboside, Methyl pentofuranoside, Di-tert-butylphenol, 3-Acethylthymine, Tetradecanoic acid, 1-Tridecene, n-Hexadecanoic acid methyl ester, n-Hexadecanoic acid, 1, E-11, Z-13-Octadecatriene, 1-(4-Bromobutyl)-2-piperidinone, 1-Flourodecane, and Palmitic acid β-monoglyceride. These arrays of biological compounds are known to function as antioxidants, antimicrobials, and energy sources. This emphasises that the syrup will be used as food and medicine for the human healthcare system.
Keywords: *fruit, GC-MS, health, phytochemical, syrup, Vitex doniana*

Introduction

The health-promoting properties of plant-based foods have largely been attributed to their wide range of phytochemicals, of which many are present at relatively high levels (Qorbani et al., 2022). Vitex is one of the genera in the family Lamiaceae. Though it was formerly under the family Verbenacea, the most known among Vitex species are *Vitex agnus-castus* L, *Vitex cymosa Bertero* ex Spreng, *Vitex peduncularis* Wall. Ex Schaner, *Vitex negundo* L, *Vitex rotundifolia*, and *Vitex trifolia* L. (Kamal et al., 2022). However, Vitex doniana (black plum) is a potential plant in this family with lots of pharmacological and health benefits, but it seems to be underutilized. *V. doniana* is a tree crop commonly found in the savannah regions of tropical Africa. The plant is used for the management and prevention of many diseases that affect humans (Agbafor and Nwachukwu, 2011). A dyestuff for textile material has also been produced from the pulp extract (Aiwonegbe et al., 2017; Tadzabia et al., 2013). The fruit is rich in vitamins, minerals, and sugar (Vunchi et al., 2011). Several countries in the world have researched and adopted traditional means of solving health issues because of the more active compounds derived from plants with medicinal values. Because of the advantages traditional herbal remedies have over conventional drugs and therapies, the majority of

countries in the world now recognise the natural resources in medicine, and it is of vital importance to expand the knowledge and research on using traditional medicine (Nautiyal and Dubey, 2021). Plants have been employed for the treatment of several diseases, not only because they are common and easily accessible but also because of their natural sources of bioactive compounds that cannot be overlooked. Despite this, natural compounds are still the primary source of modern drug discovery (Patil and Jadhav, 2020). This study aims to evaluate the GC-MS and chemicals of a syrup prepared from the fruit of *V. doniana*.

Materials and Methods

Collection and preparation of sample

Ripe fruits of *Vitex doniana* (black plum) were collected in September 2023 from the forest and sorted. The healthy-looking sorted fruits were washed with water and taken for processing. Meanwhile, before the processing, the fruit was authenticated by a botanist (Dr. Imarhiagbe Odoligie of the Department of Biology, Edo State University, Uzairue, Edo State, Nigeria). The herbarium number is ED 4432.

Syrup preparation

The fruits were mashed gently with a mortar to avoid breaking the seeds. The breaking of the seeds when mashing will make the syrup bitter. The mashed fruits were mixed with clean water, sieved with a large-pore sieve, and again filtered with a double-muslin cloth with a small pore size that will filter all the debris. The debris-free filtrate was boiled for hours to get thick. The thick substance, which is dark in colour, is the syrup, and it has a sweet taste comparable to honey.

Preliminary qualitative chemical screening of V. doniana syrup

The chemical contents of the syrup sample were determined by the criteria of Evans (2009) and Harbone (2000).

Determination of total phenol

From the syrup, 0.2 mL was obtained and mixed with 2.5 mL of 10% Folinciocalteaus reagent and 2 mL of 7.5% sodium carbonate ($NaCO_3$). The mixture was shaken and incubated at 45 oC for 40 minutes. Using a spectrophotometer, the absorbance of the mixture was taken at 700 nm. Meanwhile, garlic acid was used as the standard.

Determination of total flavonoid

One millilitre (1 mL) of the sample was mixed with 20 ml of dilute ammonia solution, which showed a yellow colour. 1 mL of concentrated H_2SO_4 was thereafter added, and the disappearance of the yellow colour in the solution indicates the presence of flavonoid. *Determination of tannin*: To 5 mL of the sample, 20 mL of distilled water was added and stirred. The mixture was filtered, and a ferric chloride reagent was added to the filtrate for colour change. The appearance of a blue-black-green precipitate indicates the presence of tannin. *Determination of steroid*: Twenty millilitres (20 mL) of acetic anhydride were added to 1 mL of the sample and filtered. To the filtrate, 2 mL of

concentrated H_2SO_4 was added, which changed the colour of the mixture from violet to blue or green. The appearance of the blue or green colour in the mixture is an indication of the presence of steroids in the sample. *Determination of alkaloid*: One millilitre (1 mL) of the sample was mixed with 5 mL of 1% aqueous HCl and placed in a steam water bath for 2 min. The mixture was allowed to cool and filtered. 1 ml of the filtrate was treated with a few drops of Dragendorf reagent. The appearance of blue-black turbidity is an indication of the presence of alkaloids in the sample. *Determination of saponin*: One millilitre (1 mL) of the sample was mixed with 5 ml of distilled water in a test tube until frothing. The tube was then placed in a water bath regulated at 40 oC for 3 minutes. The persistence of frothing in the tube indicates the presence of saponin. *Determination of terpenoid*: One millilitre (1 mL) of the sample was mixed with 20 mL of chloroform and filtered. 3 mL of concentrated H_2SO_4 was added to the filtrate to form a layer. The formation of a reddish-brown colour at the interface indicates the presence of terpenoids in the sample.

Determination of cardiac glycosides

One millilitre (1 mL) of the sample was treated with pyridine, and a few drops of 2% sodium nitroprusside and 20% NaOH were added. A deep red colour that faded to a brownish yellow is an indication of glycosides present in the sample.

Determination of phlobatannin

The deposition of a red precipitate after boiling 1 mL of the sample with 1% aqueous HCl is proof of phlobatannin present in the sample.

Determination of anthraquinone

The Borntragers test was adopted, where 10 mL of benzene was mixed with 1 mL of sample, shaken, and filtered. Thereafter, 5 mL of a 10% ammonia solution was added to the filtrate. It was shaken, and the presence of a pink, red, or violet colour in the ammonia layer is an indication of free anthraquinone presence in the sample.

Preliminary quantitative chemical screening of V. doniana syrup

Determination of the total phenols

The total phenol content of the syrup was determined by the method of Brunner (1984). To 1 mL of the syrup, 2.5 mL of 10% Folinciocalteaus reagent and 2 mL of 7.5% sodium carbonate were added. The mixture was incubated for 40 minutes at 45 °C, then removed from the incubator and allowed to cool. The absorbance of the sample was measured at 700 nm in a spectrophotometer. Meanwhile, garlic acid was used as the standard.

Determination of saponin

The method of Brunner was adopted. 1 mL of the sample was measured into a 250-mL-capacity beaker and mixed with isobutyl alcohol. The solutions were mixed with a shaker for 4 hours, and using No. 1 Whatman filter paper, they were filtered into a beaker containing 20 mL of a 40% saturated solution of magnesium carbonate ($MgCO_3$). Filtration was repeated a second time, after which 1 mL of the sample was placed in a 50-mL volumetric flask. 2 mL of 5% iron (iii) chloride ($FeCl_3$) was added

and made up to the mark with distilled water. It was allowed to stand for 30 minutes for colour development before taking the absorbance against a blank at 380 nm (Brunner, 1984).

Determination of flavonoid

The method of Bao et al. was adopted. 0.3 mL of 5% $NaHO_3$ was added to 1 mL of the sample and allowed to stand for 5 min. After which, 0.6 mL of 10% $AICI_3$ was added and stood for 6 minutes. 2 mL of 1 M NaOH and 2.1 mL of distilled water were added to the mixture before taking the absorbance at a wavelength of 510 nm against the reagent blank. Flavonoid was expressed as mg rutin equivalent (Bao et al., 2005).

Determination of terpenoids

One millilitre (1 mL) of the sample was measured into a beaker, and 20 mL of chloroform/methanol ratio 2:1 was added, mixed properly, and allowed to stand for 15 min at room temperature. The solution was centrifuged at 3000 rpm. The precipitate was rewashed with 20 mL of a chloroform-methanol ratio of 2:1 after discarding the supernatant. The sample was centrifuged for the second time, and the precipitate was dissolved in 40 mL of SDS solution. 1 ml of 0.01 M ferric chloride was then added and allowed to stand for 30 min before taking the absorbance at 510 nm (Makkar et al., 1996).

Determination of tannin

The method of Makkar et al. (1996) was used. 1 mL of the sample was placed in a sample bottle, and 10 mL of 70% aqueous acetone was added and covered. The bottle with the content was placed in an ice bath shaker at 30 °C for 2 hours. The sample was centrifuged, and the supernatant was placed on ice. 0.2 mL of the solution was pipetted into a test tube, and 0.8 mL of distilled water was added. A standard tannin acid solution was prepared from 0.5 mg/mL of the stock and made up to 1 mL with distilled water. Thereafter, 0.5 mL of Folinciocateau reagent was added to both the sample and the prepared standard tannin solution, followed by the addition of 2.5 mL of 20% Na_2CO_3. The solutions were vortexed and incubated at room temperature for 40 minutes. The absorbance of the sample was read at 725 nm against a reagent blank concentration (Makkar et al., 1996).

Determination of steroid content

The quantity of steroid content in the sample was determined by measuring 2 mL into a conical flask and mixing it with 50 mL of pyridine. The mixture was shaken for 30 minutes, and 3 mL of 250 mg/mL of metallic copper was added and incubated for an hour in the dark. Thereafter, the absorbance was read at 350 nm against the reagent blank (Makkar et al., 1996).

Determination of glycosides

Ten millilitres (10 mL) of the sample were placed in a conical flask and mixed with 50 mL of chloroform for 1 hour by a vortex mixer. 10 mL of pyridine and 2 mL of 29% sodium nitroprusside were added and shaken vigorously for 10 min. Thereafter, 3 mL of 20% NaOH was added for colour development to brownish yellow. A standard

concentration of glycosides (Digitoxin) with a range of 050 mg/mL was prepared. The absorbance of the sample alongside the standards was read at 510 nm (Makkar et al., 1996).

GC-MS analysis of the syrup

The method as described by Ashraf et al. (2017) was adopted. The syrup sample was analysed with gas chromatography-mass spectrometry (GCMS-QP1010 Plus Shimadzu, Japan). The equipment was operated at a column oven temperature of 80 °C with an injection temperature of 250 °C, a pressure of 108.0 kPa, a column flow of 1.58 ml/min, and a scanning mass that started with m/z 40 and ended with m/z 600. The obtained components were identified by comparison of the mass spectra with standard data from the National Institute of Standards and Technology (NIST) library (Ashraf et al., 2017).

Results and Discussion

Qualitative and quantitative phytochemicals

The phytochemicals that were qualitatively screened from the syrup are phenols, flavonoids, tannins, alkaloids, saponins, terpenoids, and cardiac glycosides. Quantitatively, these chemical compounds have values of 17.437, 0.289, 19.088, 48.273, 3.139, and 26.817, respectively (*Table 1*). Both the quality and quantity of phytochemicals in *V. doniana* syrup were screened with chemical methods. The phytochemicals, which are phenols, terpenoids, steroids, alkaloids, saponins, and cardiac glycosides, have been reported to be present in the fruit pulp and leaves of V. doniana (Osuagwu and Eme, 2013; Salihu et al., 2011). Several works of literature have reported these chemicals to serve in one form or another in health care, such as the use of *V. doniana* by different cultures as an active agent for application in anti-inflammatory and anti-allergy applications (Adjei et al., 2021; Ishurd et al., 2004). Anti-viral, antimicrobial, anticancer (Aiwonegbe et al., 2018), reduction of cholesterol and bile acid (Shereen, 2011), treatment of wounds, malaria, sterility, hemorrhoids, diarrhea, and several others in health management. The emphasis on this is that quality and active drugs could be formulated from the fruits, leaves, stem bark, and any other part of *V. doniana* due to its robust metabolites. This could be justified on the basis that the standardization of natural products or herbal remedies remains one of the best ways to ensure their quality in drug production (Kunle et al., 2012).

Table 1. *Qualitative and quantitative chemical components of V. doniana*.

Chemical	Quality	Quality in Mean±SD
Phenols	+	17.437
Flavonoids	+	0.289
Tannis	-	-
Steroids	-	ND
Alkaloids	+	19.088
Saponius	+	48.273
Terpenoid	+	3.139
Cardiac glycosides	+	26.817

Notes: + means positive result, - means negative result.

Chemical compounds

Figure 1 shows the GC-MS chromatogram of the syrup, where 19 compounds were identified with a retention time that ranged from 4.727 to 24.701 min. The presence of the valuable chemicals informed the further study of the syrup for GC-MS for the detection of bioactive compounds. Glycerol, as one of the bioactive compounds identified with GC-MS, is in the class of organic compounds referred to as sugar alcohol. Its presence in the syrup could serve as an energy booster; hence, glycerol can be converted by the liver into glucose for the metabolism of cells. This compound is useful in the food industry, where it could be utilized as a sweetener and as a humectant in the formulation of some pharmaceutical products, skin lotions, and cough syrup. L-mannomethylose, another identified compound, is a deoxysugar and is used as one of the components in some antibiotics, therapeutics, and antimicrobial agents. Alison and Nicola, have reported that the presence of deoxysugars in drugs dictates the binding of receptors, efficacy improvement, and modification of glycoconjugate function (Vickman and Pohl, 2019). Literature on the excessive consumption of fructose and glucose by humans has reported several ill health implications. For this reason, much research interest has shifted to the use of non-added sugars as an alternative to substances with added sugars. This is a guide that motivated us to develop a syrup of non-added sugar from *V. doniana* fruit. The practice of this will, by several means, address the complications of health encountered in many communities around the world. Yasimine et al, reported that the alternative to substitute sugar can be derived from hemicellulose, which is found in quantity in plant-based foods such as potatoes, coconuts, and corn. Based on the chemical components we have identified from the syrup with GC-MS and the health benefits the chemicals could have on the physiological system, we are adding them with confidence to the plant-based foods Alam and co-authors have recommended (Alam et al., 2022).

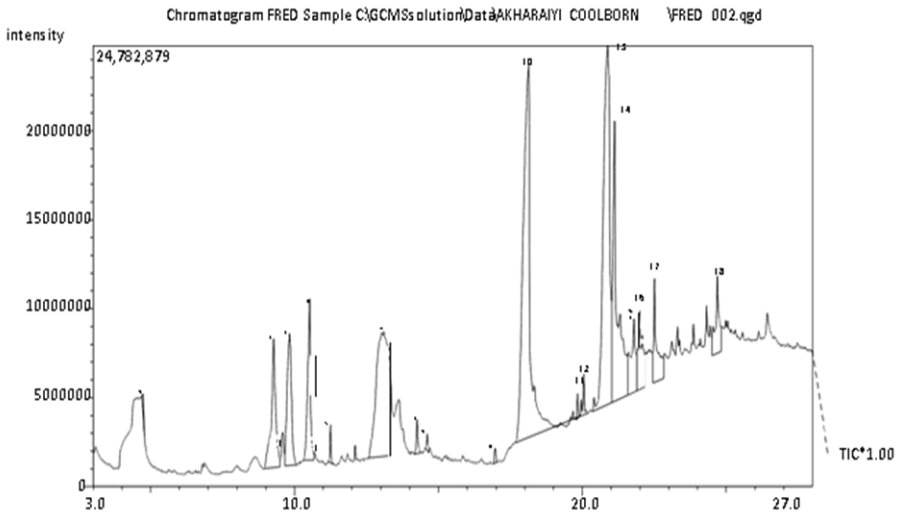

Figure 1. *GC-MS chromatogram of the syrup.*

The identified chemical compounds were confirmed based on their percentage area, percentage height, structure formula, retention time, and molecular weight. The identified chemical compounds with their relative percentage area and retention time are: glycerin (0.17%) at 4.727, L-mannomethylose 5.21% at 9.273, ethyl β-d-riboside at 9.823, and methyl pentofuranoside (4.28%) at 10.531, 3,5-Di-tert-butylphenol (0.35%) at 11.250, 3-Acetylthymine (13.05%) at 13.103, Tetradecanoic Acid (0.55%) at 14.253,

1-Tridecene (0.22%) at 14.257, n-Hexadecanoic acid methyl ester (0.20%) at 16.969, n-Hexadecanoic acid (24.28%) at 18.124, 1-Docosene (0.25%) at 19.850, Methyl cis-6-octadecenoate (0.64%), at 19.972, Erucic acid (21.68%) at 20.886, Octadecanoic acid (11.83%) at 21.120, 1, E-11, Z-13-Octadecatriene (3.11%) at 21.800, 1-4 (-Bromobutyl) -2-piperidinone (3.19%) at 22.000, 1-Flourodecane (3.11%) at 22.517, and palmitic acid β-monoglyceride (2.57%) at 24.701 (*Table 2*). 3-Acetylthymine stabilizes nucleic acid's structure because of its nitrogen content. This structure is important for DNA functions. 1-Tridecen is a natural product known for its nematicidal and ovicidal potential.

Table 2*. GC-MS chromatogram of the syrup from V. doniana.*

Peak	Compound name	Area %	Height %	Structure fornula	Retention time	Molecular weight
1	Glycerin (sugar alcohols.8)	0.17	0.71	C_3H8O_3	4.727	92
2	L-Mannomethylose	5.21	6.42	$C_6H_{12}O_5$	9.273	164
3	Ethyl .β.-d-riboside	5.31	6.60	$C_7H_{14}O_5$	9.823	178
4	Methyl pentofuranoside	4.28	8.05	$C_6H_{12}O_5$	10.531	164
5	3,5 -Di-tert-butylphenol	0.35	1.91	$C_{14}H_{22}O$	11.250	206
6	3-Acetylthymine(acyl cholines)	13.05	6.23	$C_7H8N_2O_3$	13.103	168
7	Tetradecanoic acid	0.55	1.72	$C_{14}H_{28}O_2$	14.257	228
8	1-Tridecene	0.22	0.84	$C_{13}H_{26}$	14.257	182
9	n-Hexadecanoic acid methyl ester	0.20	0.76	$C_{17}H_{34}O_2$	16.969	270
10	n-Hexadecanoic acid	24.28	18.18	$C_{16}H_{32}O_2$	18.124	256
11	1-Docosene	0.25	1.07	$C_{22}H_{44}$	19.850	308
12	Methyl cis-6-octadecenoate	0.64	0.70	$C_{19}H_{36}O_2$	19.972	296
13	Erucic acid	21.68	17.64	$C_{22}H_{42}O_2$	20.886	338
14	Octadecanoic acid	11.83	13.75	$C_{18}H_{36}O_2$	21.120	284
15	1,E-11,Z-13-Octadecatriene	3.11	3.54	$C_{18}H_{32}$	21.800	248
16	1-(4-Bromobutyl)-2-piperidinone	3.19	3.78	$C_9H_{16}BrNO$	22.000	233
17	:Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxyphosphinyl]oxy]methyl]-1,2-ethanediyl ester	3.11	4.43	$C_{37}H_{74}NO_8P$	22.517	691
18	1-Fluorodecane	3.11	4.43	$C_{10}H_{21}F$	22.517	160
19	Palmitic acid .β.-monoglyceride	2.57	3.68	$C_{19}H_{38}O4$	24.701	330

Though this compound has been detected by many authors, Valettea et al. (2003), reported that it has not been quantified in various foods, and as such, it will be a potential biomarker for the consumption of some foods such as cauliflower, fruits, nuts, and milk products. 1-Tridecen is one of the bioactive compounds we have identified from *V. doniana* syrup. The syrup could therefore be employed in traditional medicine for its value in the health care of humans (Valette et al., 2003). Erucic acid has been reported to enhance cognitive function and inhibit lactase and thrombin (Kumar and Sharma, 2022). Octadecanoic acid is a saturated fatty acid with a straight chain and can be found in animal and vegetable lipids or diets. It is a substance useful in making cosmetics, softening plastics, and hardening soaps. I, E-11, and Z-13-Octadecatriene belong to the fatty acid group. Fats are sources of energy, however, unsaturated fatty acids in dietary intake have more benefits in the human system than saturated fatty acids (Shahidi and Ambigaipalan, 2018). Hexadecanoic acid methyl ester has antioxidant, hypocholesterolemic nematicide, and antibiotic activity (Selvarani and GV, 2014). Palmitic acid has several functions in the physiological system. However, the attempt to maintain the mechanism of a steady palmitic acid concentration in the system could be altered and may lead to excessive amounts in the body tissue that could result in ill health such as hyperglycemia, increased inflammatory tone, increased ectopic fat accumulation, and dyslipidemia (Carta et al., 2017). The pharmacological importance of the syrup also lies in the functional groups to which some of the bioactive compounds belong. For example, methyl groups have been found useful in biochemical processes and play a vital role in drug design (Lian et al., 2013). It also has several roles in

cellular functions, such as the biosynthesis of proteins and the methylation of DNA (Erichsen et al., 2022). The long-chain fatty acids can serve as a substance of energy. The most known unsaturated fatty acids are omega-3, omega-6, and omega-9 (oleic acid). Omega-3 long-chain fatty acids have potency as an anti-inflammatory effect (Johnson and Bradford, 204).

Conclusion

This research specifies the several active compounds in *V. doniana* fruit syrup. Some of the bioactive compounds identified are long fatty acids, unsaturated aliphatic hydrocarbons, and methyl groups. The various bioactive compounds in the syrup emphasize that it has value as food and medicine in the human health care system. Therefore, it may be used for managing various diseases in humans and as a precursor in the food and pharmaceutical industries.

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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