

Inhibition of Bacterial Growth, Mineral, Proximate and Vitamin Contents of a Syrup Prepared from *Vitex Doniana* Fruits

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ABSTRACT

Background: This study aims to investigate the *in-vitro* antibacterial activity, mineral and vitamin compositions, proximate composition, and organoleptic properties of a syrup derived from *Vitex doniana* fruits.

Materials & Methods: *V. doniana* fruits were mashed, mixed with water, strained, and boiled to thicken the filtrate. The syrup's antibacterial activity was tested on 7 clinical and 6 American Type Culture Collection (ATCC) isolates using well-in-agar diffusion method on Mueller Hinton agar. The fruit juice underwent mineral analysis using atomic spectroscopy and mass spectrometry. Proximate composition, vitamin, and organoleptic properties of a syrup were evaluated.

Findings: Clinical *Escherichia coli* 0157:H7 and *Staphylococcus aureus* isolates were susceptible to the syrup, with inhibition zone of 25 mm each while *S. aureus* ATCC 25923 had the highest susceptibility with a 33 mm inhibition zone. The syrup showed varying minimum inhibitory concentrations (12.5-50 mg/ml) and minimum bactericidal concentrations (25-150 mg/ml) against tested bacteria. The syrup contained 18.4±0.36 mg calcium, 36.92±0.14 mg magnesium, 3.21±0.30 mg iron, 4.80±0.24 mg sodium, and 43.56±1.05 mg potassium as mineral composition per 100 g. Although the prepared syrup had higher calcium, magnesium and iron values prepared to the commercial sample, there was no significant difference between the two. Proximate composition analysis revealed moisture content was measured at 20.83±1.08% moisture content, pH=4.76, 0.20±0.01% crude fiber, 2.40±0.35% crude protein, 3.18±1.12% ash, 0.62±0.24% crude fat, and 76.70±0.16% carbohydrate levels in the syrup. Significant difference was only found in ash and carbohydrate values, with the prepared sample showing higher levels. The syrup exhibited higher vitamin content, including vitamin C, B₁, B₂, B₆ and A, compared to the commercial sample. In terms of organoleptic properties, the prepared syrup scored slightly better in taste, flavor, and overall acceptability (0.18%) compared to the commercial product.

Conclusions: Based on these finding, the syrup derived from *V doniana* shows potential as a nutrient food product with antimicrobial properties. It could be used in healthcare, industrial applications (such as preservatives or sweeteners), and as a base for pharmaceutical formulations. Furthermore, the syrup may find applications in the confectionery, bakery industries, and traditional medicine.

Keywords: Antibacterial agent, syrup, health importance, Nigeria.

CITATION LINKS

[1] Benny PJ, Shibumon G. A review on the medicinal significant of ... [2] Sahreen S, Khan MR, Khan RA. Comprehensive assessment of phenol ... [3] Aberoumand A, Deokule SS. Element's evaluation of some edible v ... [4] Akhtar S, Naz S, Tuseef SM, ... [5] Akharaiyi FC, Oso BA, Boboye BE, ... [6] Adewumi OA, Ugoanyanwu V, Osaghae OE. ... [7] Akharaiyi FC, Boboye BE, Akinyosoye FA, Oso BA. Antioxidant, ph ... [8] Gidado A, Daja A, Kassim ZM, Idris A, Audu M. Free Sugars and ... [9] Egharevba HO, Ocheme OE, Ugbabe G, Abdullahi MS, Iliya I, Okhal ... [10] Pare DH, Hilou A, Quedraogo N, Guennes S. Ethnobotanical study ... [11] Nwachukwu E, Uzueto HO. Antimicrobial activities of leaf of Vit ... [12] Agbafor KN, Nwachukwu N. Phytochemical ... [13] Lagnika L, Amoussa M, Adjovi Y, Sunni A. Antifungal, antibacter ... [14] Holt JG, Krieg NR, ... [15] Moradian H, Bazargani A, Rafiee A, Nazarialam A. *In vitro* compa ... [16] Irobi ON, Moo-Young M, Anderson WA, ... [17] Akharaiyi FC, Oso BA, Boboye BE, ... [18] Pohl P, Dzimittrowicz A, Jedryczko D, Szymezycha-Madeja A, Welna ... [19] Methods of Analysis of AOAC International (18th ed.). Arlington ... [20] Jacop MB. The chemical ... [21] Official Methods of Analysis of ... [22] The British Pharmacopedia ... [23] Kolawole SO, Kolawole OT, Akanji MA. Effects of aqueous extract ... [24] Akharaiyi FC, Ezeanya-Bakpa CC, Otumala SO. Inhibition of methi ... [25] Bouabdelli F, Djelloul A, Kaid-Omar Z, ... [26] Tavares LS, Silva CFS, Souza VC, ... [27] Tiburski JH, Amanuri R, Rosires D, Ronoel L, de Oliveira G, Sid ... [28] Akharaiyi FC, Boboye B, Adetuyi FC. Antibacterial, phytochemica ... [29] Vunchi MA, Umar AN, King MA, ... [30] Aiwonegbe AE, Iyasele JU, Momodebe RO. Characterization of a na ... [31] James D. Abu. Development of a sweetener from black ...

Introduction

The increasing global focus on plant derivatives and remedies derived from fruits and seeds reflect the growing recognition of the significance of plants in the healthcare system. This is driven by the limitations of conventional medicine in treating both microbial and non-microbial diseases. The use of plant based products to address health challenges has transitioned from ancient to modern societies, establishing a remarkable arsenal in drug formulation for their utilization in the treatment and prevention of diseases.

Ancient societies recognized the therapeutic properties of fruits, beyond their nutritional value, in the treatment of certain ailments. Scientific advancements in biology have enhanced our understanding of the nutritive qualities and physiological benefits of fruits, highlighting their importance in both food and medicinal resources [1]. One study in 2014 revealed that the *in-vivo* protection against oxidative stress can be attributed to the presence of antioxidants in fruits [2]. Fruits as unique food sources are rich in essential mineral elements such as magnesium, calcium, and potassium. The consumption of these minerals among others is associated with a reduced risk of cancer, aging, cataracts, and cardiovascular diseases [3]. Akhtar et al. [4]; have reported that fruits as significant sources of nutrients and play pivotal roles in human diets, providing essential minerals, vitamins, and other valuable constituents necessary for maintaining human health.

The recognition of the health benefits of consuming fruits, as supported by modern science has led many individuals to incorporate fruits into their daily diets or consume them in the form of juice or syrup [5]. The Interest in these fruits stems from the fact that the plants producing them, have documented leaves, stem bark, or roots containing synthesized secondary metabolites with anti-

microbial, anticancer, anti-tumor, anti-helminthic, and anti-parasitic effects among others. The importance of plant-derived products from fruits in health maintenance has prompted numerous research efforts that have yielded fruitful results in disease prevention and treatment. The efficacy of these plant-derived products can be attributed to their mineral, chemical, and vitamins contents, which contribute to their therapeutic value. Some fruits due to their chemical, vitamin, and mineral composition, have been found to support growth and overall health [6]. For instance, *Spondias mombin* has been extensively researched for its antimicrobial, antioxidant, physicochemical, and mineral properties [7]. These properties are attributed to the presence of chemicals such as phenolics, steroids, tannins, saponins, alkaloids, terpenoids, as well as physicochemical components like protein, fat, and minerals, including sodium, magnesium, calcium, iron, zinc, among others [7]. Fruits contain minerals, vitamins fibers, and various plant components that contribute to maintaining overall bodily health. Regular consumption of fruits and vegetables is associated with a reduced risk of numerous diseases [8].

For more emphasis and details of the plant in this research study which is *Vitex doniana*, typically reaches heights of 10-12 m. It is widely distributed in Africa, especially in Ghana, Burkina Faso, Nigeria, and Cameroon. The tree produces small white flowers and green fruits that turn purplish-black when ripe. The woody seeds are spherical, with an approximate height of 1.5–20 cm and a width of 1.0–1.2 cm [9]. The raw fruits serve as appetite suppressants for farmers and hunters, and mulching with *V. doniana* has been found to improve land fertility and nitrogen fixation. The tree has diverse uses, including medicinal applications, timber production, and providing housing for bees in honey production [10]. Egharevba *et al.* [9]

conducted a research study confirming *V. doniana* leaves in managing diabetes, reducing high blood pressure, and treating oedema, swellings and ulcers. Essential phytochemicals, balsam resin, and carbohydrates have been identified and screened from various parts of the plant [11-13]

Objectives: Some conventional drugs known for the cure and prevention of illnesses caused by pathogenic bacteria burden some rural and urban dwellers because of the high purchase cost. Therefore sourcing for effective alternatives for their susceptibility is of importance. Both clinical and type culture collection strains that commonly cause diseases were studied with *V. doniana* fruit syrup as an alternative to conventional drugs. The study also entails the importance of the syrup's mineral, proximate composition, vitamins, and bacterial inhibition properties.

Materials and Methods

Sample collection and Preparation

Mature fruits of *V. doniana*, commonly known as black plum, were gathered from the forest and carefully sorted. Only visually healthy fruits were selected for further processing, and they were thoroughly washed with water.

Syrup preparation

The fruits were carefully macerated using a mortar to prevent seed breakage, as this enhances the quality of the resulting syrup. The mashed fruits were subsequently combined with clean water and strained through a coarse sieve, followed by a double muslin cloth with a finer pore size to remove any remaining debris. The resulting debris-free filtrate was then boiled for several hours until it thickened. The dark-colored, thickened substance is the syrup, which exhibits a sweet taste likened to honey.

Tested bacteria species

Seven clinical bacterial isolates namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica Serotype Typhi*, *Bacillus cereus*, *Escherichia coli* 0157:47, *Escherich-*

ia coli, and *Staphylococcus aureus*, were obtained from consented hospitalized patients by collecting urine, feces and wound swabs. The collected samples underwent serially diluted and 1 ml of each sample was plated on specific agar media including Mannitol salt agar (MSA), Eosin methylene blue agar (EMB), Nutrient agar, MacConkey agar (MCA), and Nutrient agar (NA). The plates were incubated at 37 °C for 24 h. Subsequently, the grown bacteria on the plates were sub cultured and purified on nutrient agar plates. Multiple identification methods were employed to confirm the identity of the isolates. The pure, characterized and identified bacterial species were preserved on an agar slants for further evaluation. Additionally, six American Type Culture Collection strains were used, namely *Klebsiella pneumoniae* ATCC 49619, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 10749, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 35218, and *Staphylococcus aureus* ATCC 25923. Prior to use, these strains were sub-cultured for purification, and Gram staining was performed to confirm cell morphology and Gram reaction. The purified bacterial species were characterized and identified based on the criteria described by Holt et al. (1994) [14]. Before conducting antibacterial activity assays, the bacterial species were cultured in test tubes for 24 h. The resulting turbid cultures, indicating bacterial growth were diluted with sterile distilled water to match the McFarland's standard of 10⁶ colony-forming units per milliliter (CFU/ml).

Determination of the antibacterial activity of *V. doniana*

The antimicrobial activity was assessed using the well-in-agar method. Mueller Hinton agar was prepared following the manufacturer's instructions. A 24-hour broth culture of each tested bacterial species was adjusted to match the McFarland standard (10⁶) [15].

Subsequently, 1 mL of each bacterial culture was pipetted onto a sterile plate and overlaid with molten Mueller Hinton agar. The plate was swirled to ensure an even distribution of cells. After allowing the seeded Petri-plates to solidify at room temperature for 2 h, wells were created in the agar to accommodate the syrup. Using a micropipette, 0.5 mL of the syrup was introduced into each well. The plates were then incubated aerobically at 37 °C for 24–48 h. Following incubation, the zones of inhibition formed around each bacterial specie were measured and reported as the level of sensitivity ⁽¹⁶⁾.

Determination of minimum inhibitory concentration (MIC)

This method serves as a fundamental approach for evaluating the antimicrobial activity of substances against microorganisms. The syrup concentrations used to determine the MIC ranged from 5% to 80%. In a sterile manner, 1 mL of an overnight culture of the tested bacteria, adjusted to match the McFarland's standard was combined with 1 mL of each prepared syrup concentration and 8 mL of sterile Mueller Hinton broth in separate test tubes. These tubes were then incubated at 37 °C for 24 h to observe for turbidity or clarity. The tube with the lowest syrup concentration that exhibited a clear visual appearance to the naked eye was the MIC value for each tested bacteria ⁽¹⁷⁾

Determination of minimum bactericidal concentration (MBC)

The tubes that displayed a clear visual appearance to the naked eye were selected and gently shaken. Subsequently, 1 mL of the contents from each tube was drawn using a sterile pipette and plated on freshly prepared plate count agar. The cultured plates were then incubated at 37 °C for 24–48 h. The plates without any bacterial growth at the lowest concentration were identified as having a bactericidal effect, while plates with minimal bacterial growth were consid-

ered to have bacteriostatic effect ⁽¹⁷⁾

Mineral contents determination

The method of Pohl et al. 2016 ^[18] was used for the analysis. The syrup sample was digested using the wet ashing method, and quantification was carried out using atomic spectroscopy and mass spectrometry [ICP-MS, Spectro Analytical Instruments GmbH]. The syrup sample was aspirated into an air-acetylene flame to cause evaporation of the solvent and also to vaporize the free metalation (atomization). To determine Calcium Magnesium, Ion, Sodium and Potassium, lotions (La (III) ions was used as an ionization buffer and was added to both the syrup and standard solutions. This was done to overcome chemical interferences in the flame during the process. The analyses were conducted three times, and the results were reported in mg/100 g.

Determination of Proximate composition Moisture determination

We followed the AOAC methods 997.15 and 990.08, (2005) ^[19] a criterion was adopted. 20 ml of the syrup was measured into a clean crucible with a known weight and placed in a regulated oven at 650 °C for 1 h. After which, it was removed and weighed. This was repeated until a constant weight was achieved.

Calculation

$$\text{Moisture content: } \frac{(W_2 - W_1) - (W_3 - W_1)}{W_2 - W_1} \times 100$$

Where W1 = weight of empty crucible

W2 = weight of crucible with sample

W3 = weight of crucible with dry sample

Determination of ash contents

We utilized the method outlined by AOAC (2005) ^[19]. A volume of 20 mL of the syrup was measured and placed in a dry crucible. The crucible was then heated on a hot plate to remove organic matter and subsequently transferred to a muffle furnace, where it was exposed to a temperature of 600 °C for du-

Table 1) Antibacterial activity of *V. doniana* in comparison with Honey

Bacteria species	Inhibition zones (mm)	MIC (mg/mL)	MBC (mg/mL)
<i>Klesiella pneumoniae</i>	20	12.5	50
<i>Klebsiella pneumoniae</i> ATCC 49619	25	12.5	25
<i>Pseudomonas aeruginosa</i>	19	50	150
<i>Pseudomonas aeruginosa</i> ATCC 27853	25	50	100
<i>Salmonella enterica serotype Typhi</i>	18	50	100
<i>Salmonella typhi</i> ATCC 10749	21	12.5	50
<i>Bacillus cereus</i>	20	50	50
<i>Bacillus cereus</i> ATCC 14579	22	12.5	25
<i>Escherichia coli</i> 0157:H7	25	50	100
<i>Escherichia coli</i> ATCC 35218	31	25	50
<i>Staphylococcus aureus</i>	25	50	50
<i>Staphylococcus aureus</i> ATCC 25923	33	25	50

Table 2) Composition of proximate in *V. doniana* syrup (%)

Sample	Moisture	pH	Crude fibre	Crude protein	Ash	Crude fat	Carbohydrate
Prepared	20.83±1.08	4.76	0.20±0.01	2.40±0.35	3.18±1.12	0.62±0.24	76.70±0.16
Commercial	20.73±1.14	4.86	0.20±0.02	2.33±0.16	2.27±0.18	0.60±0.14	74.68±0.11

Table 3) Organoleptic properties of *V. doniana* syrup (%)

Samples	Taste	Flavor	Color	Overall acceptability
Prepared	8.16	8.10	7.62	7.24
Commercial	8.09	8.05	7.62	7.18

ration of 5 h. Following the heating process, the content was cooled in a desiccator and weighed.

$$\% \text{Ash} = \frac{(\text{Crucible's weight} + \text{ash}) - (\text{Empty crucible's weight}) \times 100}{\text{Weight of sample}}$$

Determination of crude fat

According to the criteria established by AOAC [19], A 20 mL portion of the syrup was accurately measured and transferred into a fat-free extraction thimble, which was then tightly sealed. Subsequently, the thimble was placed in an extractor, and a reflux condenser was attached to it. Meanwhile, a dried 250 ml Soxhlet flask was filled with ¾ of its volume with petroleum ether.

The entire set up was then placed inside a muffle furnace and maintained for a period of 6 h, with continuous flow of tap water aiding in the condensation of the petroleum ether. The extraction process was carried out for 6 h within the thimble, after which it was carefully removed. The flask was then taken out and transferred to an oven regulated at 65 °C for 4 h. Following this, the flask was removed from the oven, allowed to cool and subsequently weighed.

Determination of crude fiber

In accordance with the standards set by AOAC [19], syrup was carefully measured in a 500 mL beaker. Next, 200 mL of preheated

1.25% H₂SO₄ was added to the beaker and placed in a regulated digestion apparatus. The sample was refluxed for 30 min, and filtered using No 1 Whatman filter paper. The obtained residue was boiled in hot distilled water until a neutral filtrate was obtained. The residue was taken into a clean crucible and dried for 24 hours at 65 °C. The combined weight of the sample (labeled as "A") was recorded. Subsequently, the crucible with the sample was subjected to a furnace at 600 °C, it was re-weighed (labeled as "B").

$$\% \text{ Crude fiber} = \frac{\text{DWR} - \text{WR}}{\text{SW}} \times 100$$

SW

DWR denotes WR weight of the residue after ashing, and SW represents weight of the sample.

Crude Protein determination

The Kjeldahl Nitrogen method, described in AOAC (2005) [19] was adopted. 10 mL of concentrated H₂SO₄ and 1 Kjeldahl catalyst catalyst tablet were added. The mixture was then digested for 4 h. Following, the sample was cooled and transferred thoroughly. Using a pipette, 5 ml of 40% NaOH was added. In a conical flask a mixture of 10 mL boric acid mixed with indicator solution and placed at the receiving top of the condenser. The resulting sample solution was then titrated against a 0.01 N concentration of HCL

$$\% \text{ Nitrogen} = \frac{14 \times \text{VA} \times 0.1 \times \text{W} \times 100}{100 \times 100}$$

VA represents the volume of used acid, while W represents the sample's weight.

The percentage crude protein = Nitrogen × 6.25.

Carbohydrates determination

Determination of the syrup sample's carbohydrate content was by difference. This was carried out using the calculation by difference as described by AOAC method (AOAC 2016) [20]. The sample carbohydrate content was estimated by subtracting the total percentage of other components which are crude protein, crude fat, fibre, moisture, and ash from 100 using the calculation below

$$\text{Carbohydrate (\%)} = 100 - (\% [\text{crude pro-$$

tein, crude fat, fibre, moisture and ash])

Determination of vitamins

The method described by Jacobs (1999) [21] was used for the determination of vitamins A and B₁. The titimetric method was used to determine vitamin C. The British pharmacopedin (1988) [22] was employed to determine B₂ and B₆ by the spectrophotometric methods

Organoleptic properties of V. doniana fruit syrup

To assess the sensory or organoleptic properties of the syrup, the same volume 20 mL was measured and placed in clean plates. A panel constituting of nine individuals, who regularly consume the sample, was selected for the evaluation. The evaluated qualities of the syrup were taste, flavor, color, and overall acceptance. The parameters of preference on a 9-point scale were, disliked slightly (1), disliked moderately (2), disliked very much (3), disliked extremely (4), neither liked nor a disliked (5), liked slightly (6), liked moderately (7), Liked very much (8), and liked extremely (9). The evaluation was repeated three times, changes were made in scores, if necessary. Panelists were provided with drinking water at intervals to clean palates.

Data statistical analysis

The data obtained from the experiment were presented (ANOVA). Pairwise mean comparison were conducted using the least significant difference (LSD) test to determine significant differences among treatment doses at 95% level of confidence. The values obtained were considered statistically significant at ($P < 0.05$).

Findings

The syrup exhibited significant bacterial inhibition, with inhibition zones ranging from 18 to 33 mm. clinical *Salmonella Enterica Serotype Typhi* isolate showed an inhibition zone of 18 mm, clinical *Escherichia coli 0157. H7* isolate and clinical *Staphylococcus aureus* isolates were the most susceptible with inhi-

bition zones of 25 mm each. The Remaining bacteria displayed inhibition zones between 19 and 20 mm. Among the America Type Culture, *Salmonella typhi* ATCC 10749 exhibited the least inhibition with a zone of 21, while *Staphylococcus aureus* ATCC 25923 was the most susceptible with an inhibition zone of 33 mm. *Klebsiella pneumoniae* ATCC 49619 and *Pseudomonas aeruginosa* ATCC 27853 both with inhibition zones of 25 mm. *Salmonella typhi* ATCC 10749 and *Bacillus cereus* ATCC 14579 were also susceptible with inhibition zones of 21 mm and 22 mm respectively. Overall, the Type cultures demonstrated greater susceptibility to the syrup than the clinical isolates. Despite the differences in inhibition on clinical *Klebsiella pneumoniae* isolate and *Klebsiella pneumoniae* ATCC 49619, the minimum inhibitory concentration (MIC) of the syrup was effective at 12.5 mg/mL. Similarly, the MIC on clinical *Pseudomonas aeruginosa* isolate and *Pseudomonas aeruginosa* ATCC 27853 was effective at 50 mg/ml. The syrup MIC on clinical *Salmonella Enterica Sero-type Typhi* isolate and *Salmonella typhi* ATCC 10749 were 50 and 12.5 mg/ml respectively. For clinical *Bacillus cereus* isolate and *Bacillus cereus* ATCC 14579, the MIC were 50 and 12.5 mg/mL respectively. Clinical *Escherichia coli* isolate, *Escherichia coli* ATCC 35218, clinical *Staphylococcus aureus* isolate and *Staphylococcus aureus* ATCC 25923 displayed MIC of 50 and 25 mg/mL respectively. The minimum bactericidal concentration (MBC) of the syrup exhibited activity against the tested bacterial species at concentrations ranging from 25 and 150 mg/mL. Notable, despite the MIC of 12.5 mg/mL for clinical *Klebsiella pneumoniae* isolate and *Klebsiella pneumoniae* ATCC 49619 as well as clinical *Pseudomonas aeruginosa* isolate and *Pseudomonas aeruginosa* ATCC 27853 at 50 mg/mL, the MBC values for these bacterial were 50, 25, 150 and 100 mg/ml respectively. The MBC for *Salmonella typhi* ATCC 10749, clinical *Bacillus cereus* isolate,

Escherichia coli ATCC 35218, clinical *Staphylococcus aureus* isolate, and *Staphylococcus aureus* ATCC 25923 was 50 mg/mL. However, the MBC for *Bacillus cereus* ATCC 14579 and clinical *Escherichia coli* 0157:H7 isolate was 25 and 100 mg/ml respectively (Table 1).

Figure 1 displays calcium, magnesium, iron, sodium and potassium contents of the prepared fruit syrup and were determined as 18.4 ± 0.36 , 36.92 ± 0.14 , 3.21 ± 0.30 , 4.80 ± 0.24 and 43.56 ± 1.05 mg/100 g respectively, the commercial syrup exhibited calcium, magnesium, iron, sodium and potassium values of 18.3 ± 0.42 , 35.74 ± 0.21 , 3.14 ± 0.16 , 4.85 ± 0.18 and 45.36 ± 0.34 g respectively. The prepared syrup's calcium, magnesium, and iron contents were more in value than the commercial sample. Ironically, the commercial syrup sample's sodium and potassium were more in value than the prepared sample. However, the mineral contents of the prepared syrup were comparable with the commercial syrup as the values were similar without a significant difference.

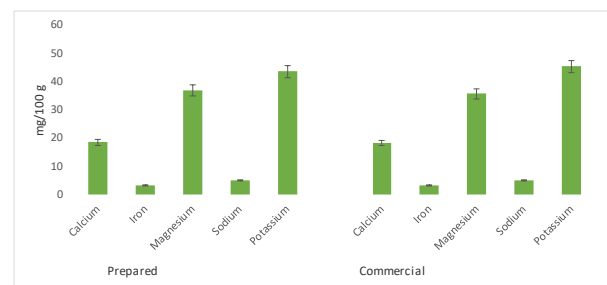


Figure 1) Mineral contents of the *V. doniana* syrup (mg/100)

Table 2 expressed the proximate composition of the syrups. The values include moisture content ($20.83 \pm 1.08\%$), pH (4.76), crude fiber ($0.20 \pm 0.01\%$), crude protein ($2.40 \pm 0.35\%$), ash ($3.18 \pm 1.12\%$), crude fat ($0.62 \pm 0.24\%$) and carbohydrate ($76.70 \pm 0.16\%$) for the prepared syrup, the commercial syrup exhibited $20.73 \pm 1.14\%$ for moisture, 4.86 for pH, $0.20 \pm 0.02\%$ for

crude fiber, $2.33 \pm 0.16\%$ for crude protein, $2.27 \pm 0.18\%$ for ash, $0.60 \pm 0.14\%$ for crude fat and $74.68 \pm 0.11\%$ for carbohydrate. There was no significant difference in moisture, pH, crude fire protein and fat contents of both the prepared and commercial sample. However, a significant difference was recorded in the ash and carbohydrate values where the prepared sample was higher in value with 0.91% and 2.02% for ash and carbohydrate respectively (Table 2).

Figure 2 illustrates vitamin C (8.92 ± 1.6 mg/100 g), vitamin B₁ (23.14 ± 1.3 mg/100 g), vitamin B₂ (9.85 ± 0.24 mg/100 g), vitamin B6 (25.35 ± 0.46 mg/100 g) and vitamin A (0.77 ± 0.03 mg/100 g) for the prepared syrup. On the other hand, the commercial syrup, vitamin C was 8.75 ± 1.4 mg/100 g, vitamin B₁ was 20.07 ± 0.2 mg/100 g, vitamin B₂ was 8.16 ± 1.3 mg/100 g, vitamin B6 was 26.42 ± 0.23 mg/100 g and vitamin A was 0.83 ± 0.11 mg/100 g.

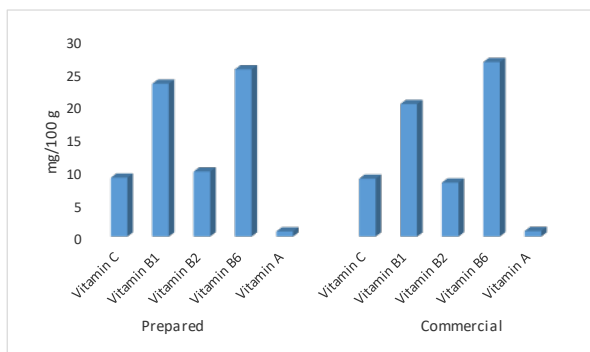


Figure 2) Vitamin contents of *V. doniana* syrup (mg/100g)

The organoleptic properties of the fruit syrup are presented in table 3. The values for taste, flavor, color and overall acceptability were 8.16% , 8.10% , 7.62% and 7.24% respectively for the prepared samples. Similarly, the commercial sample values were 8.09% , 8.05% , 7.62% and 7.24% for taste, flavor, color and overall acceptability respectively. Although the prepared sample received higher rate, than the commercial sample, there were significant differences

observed between the samples.

Discussion

Various diseases are caused by medications treatment, the exploration of alternative therapies through traditional medicine remains crucial. Frequently, conventional medicines, additionally, their high cost poses significant challenges for both rural and urban populations more, personally, is readily acceptable, exhibits minimal or no side effects, and is not easily surpassed by bacterial resistance due to the synergistic components utilization-based carries in reprioritizing health care diseases continually preserved where it is difficult to acquire. The use of plant remedies for managing bacterial diseases is mostly practiced by traditional healers and will be maintained in the traditional healthcare system for the curing and prevention of diseases. This becomes of importance and validity because the medicinal plants known for therapy are within reach to the urban and rural areas where the use of folklore in health care system delivery is a priority.

Scientific research elucidates how plants possess healing properties instigated [23-24]. The use of medicinal plants has been of importance as alternatives for the healing of various diseases because of their reliability and stability more than orthodox medicine. The valuable antimicrobial activities of medically important plants are been increasingly reported world widely to address the occurrence of pathogenic microorganisms that have multiple means of resisting drugs [25-26]. Using fruits in disease management is only 2.1% compared to its counterparts of tubers at 12.8% , leaves at 10.6% , bark at 13.7% , and roots at 55.3% [27]. Meanwhile, the majority of fruits have impressive disease healing and preventive potentials based on their compositions of vital antioxidants, trace elements, phytoconstituents, and vitamins.

The antimicrobial activity exhibited, which

displays varying inhibition spectra bacterial, could be attributed to the contained phytochemicals. The collective action of these un-separated phytochemicals could be responsible for the observed high antibacterial potency. This implies that the syrup may, in some instances, prove superior to purified antibiotics and, potentially contributing to illness [28]. Plant products in bacterial growth inhibition are an important value for the prediction of effective drugs or herbal remedies that could be used to manage microbial infections mostly by those who could accept their esteemed usefulness for alternative medicine.

Gram-negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* pose global health treats due to their high resistance to commonly used antibiotics in certain regions worldwide. The infection rates of these bacteria, among others, continue to escalate daily, emphasizing the necessity for alternative therapies. The inhibition of these bacteria species with the syrup was between 18 mm to 25 mm which is therapeutically significant extent to be certified as an antibacterial agent. No existing literature has shown inhibition higher than what has been reported in this research from either solvent extracts of the leaf stem bark or fruits of *V. doniana*. MIC of the syrup at a concentration of 12.5 mg/mL and MBC at a concentration of 25 mg/mL on some Gram-negative bacteria in this study investigation is an aspect to consider for further screening of the syrup for more medical-related indices for its certainty in therapeutic value. The pH and the acid content of the syrup could be the strong support for its antibacterial potency.

The least inhibition of clinical *Salmonella Enterica Serotype Typhi* isolate and *Salmonella typhi* ATCC 10749 with the syrup could be resulting from hash condition impacted on the bacteria species with previous drug treatments or patting way to antibiotic resistance. The high susceptibility of clinical *E. coli* 0157:

H7 isolate and *Staphylococcus aureus* ATCC 25923 could be a result of these species being wakened with previous drug treatment or the combination of the syrup metabolites working in concert on the organisms than others. The more susceptibility of the ATCC bacteria species than the clinical isolates could be the differences in their native areas, the conditions at which they were isolated from patient, and the various conditions to which they have been exposed, such as drugs, storage period, and temperature.

Significant differences were not noted in the values of minerals, vitamin contents, proximate contents, and organoleptic properties between the prepared and commercial syrups. The reason for this could be that the commercial product was obtained from the same locality where geographical conditions could not have exerted any effect on the fruits. It could also be traced to the process method used which was not different from the adopted normal traditional process of the syrup. The mineral and vitamin contents of the syrup are all health benefits besides its antimicrobial potential; hence, they help in bone formation, convert food into energy, buster the immune system, cellular damage, and wound healing. During infections from these pathogens in man, there might be a risk of blood shortage and other malfunction of the body system could emanate hence the amounts of calcium, magnesium, sodium, and potassium in the syrup could help to ameliorate these malfunctions if used. The vitamin contents are also of great value in as much the roles they play in the human body necessitate their requirement. Therefore, the contained minerals and vitamins remain a fact to be comprehended in this research hence these two vital elements could in combination play many roles in the body system. However, the contained minerals and vitamins are under the permissive level for both infants, young and old of any feminine gender even if consumed on daily bases

as food or supplement. The sample's quality in antimicrobial, mineral, and vitamins, could suggest it is an antioxidant, industrial material for preservatives, sweetener, or a base for pharmaceutical formulations in the pharmaceutical, confectionary, bakery industries, and traditional medicine

The mineral contents derived from the syrup sample were lower than those reported from the fruit pulp by Vunchi et al. (2011) [29], Ai-wonegba et al. (2018) [30]. The higher values of the fruit pulp than the processed fruits syrup could be resulting in the effect of temperature and processing methods that were different. Though the values of nutrition and energy of foods are based on proximate analysis, it only makes a known insight into the quality of the food. The proximate contents of fruit pulp as reported by Vunchi et al. (2011) [29] was also higher than what was analyzed from the fruit syrup except for carbohydrate. The higher carbohydrate value in the syrup could be a result of the heat applied during processing which makes the sugar content to be more concentrated as water activity decreased. However, significant difference ($P > 0.05$) was not reported in the proximate composition of the report of James, (2002) [31] on fruits syrup from *V. doniana* except in carbohydrate content where our report showed no difference as being significant ($P < 0.05$) in only carbohydrate. This difference could result from the plant species which were not in the same geographical location as Nigeria. Several researchers have reported differences in mineral and vitamin contents of either *V. doniana* fruit or its syrup. The fact remains clear that the differences could either be resulting from the regional environmental factors and the age of the tree which produces the fruits.

Outside other parameter values of the fruit syrup which are very essential, the crude protein content of the sample is high and could be a good source of protein for adults and children as well as those that are deficient in protein.

Conclusion

The syrup demonstrated notable antibacterial activity that might hold value in traditional medicine for treating various pathogenic bacterial infections. This syrup could, as well as its mineral and vitamin contents, the syrup can be postulated to serve as an antioxidant, an industrial preservatives, a sweetener, or a base for pharmaceutical formulations in the pharmaceutical industries, confectionery, bakery industries, and in the domain of traditional medicine.

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References

1. Benny PJ, Shibumon G. A review on the medicinal significant of common fruits. *Int J Biomed Res and Analy.* 2010;1(2):60-64
2. Sahreen S, Khan MR, Khan RA. Comprehensive assessment of phenolic and antiradical potential of *Rumex hastatus* D Don. *Roots. MMC Compl Alter Med.* 2014;14:47
3. Aberoumand A, Deokule SS. Element's evaluation of some edible vegetables and fruits of Iran and India. *Asian J Agric Sci.* 2010;2(1):35-37
4. Akhtar S, Naz S, Tuseef SM, Mahmood S, Nasir M, Ahmad A. Phytochemical attributes and heavy metal content of mangoes (*Magifera indica*) cultivated in different regions of Pakistan. *Pak J Bot.*

- 2010;42(4):2691-2702
5. Akharaiyi FC, Oso BA, Boboye BE, Akinyosoye FA. Bacterial growth inhibition properties and in vivo antioxidant effect of *Spondias mombin* fruit juice. *Romanian Archiv Microbiol and Immunol.* 2017;76(1):27-35
 6. Adewumi OA, Ugoanyanwu V, Osaghae OE. Pharmacological activities of (*Gongronema latifolium*, *Vernonia amygdalina* and *Vitex doniana*): A review. *J Pharmacog and Phytochem.* 2022;9(4):1822-1828
 7. Akharaiyi FC, Boboye BE, Akinyosoye FA, Oso BA. Antioxidant, physicochemical and mineral evaluations of *Spondias mombin* crude fruit juice. *Acta Biolog Szegediensis.* 2016;60(2):171-176
 8. Gidado A, Daja A, Kassim ZM, Idris A, Audu M. Free Sugars and Fructan Contents of Commonly Consumed Fruits of Maiduguri Metropolis North East Nigeria. *J Food Process Technol.* 2018;10: 770.
 9. Egharevba HO, Ocheme OE, Ugbabe G, Abdullahi MS, Iliya I, Okhale ES *et al.* Phytochemical screening and antimicrobial studies of methanol, ethyl acetate and hexane extracts of *Vitex doniana*, Sweet. (stem bark and leaf). *Nature and Sci.* 2010;8(8):177-185
 10. Pare DH, Hilou A, Quedraogo N, Guennes S. Ethnobotanical study of medicinal plants used as antiobesity remedies in the nomad and hunter communities in Burkina Faso. *Medicines.* 2016;3(2):1-24
 11. Nwachukwu E, Uzueto HO. Antimicrobial activities of leaf of *Vitex doniana* and *Cajanus cajan* on some bacteria. *Researcher.* 2010;2:1-11.
 12. Agbafor KN, Nwachukwu N. Phytochemical analysis and antioxidant property of leaf extracts of *Vitex doniana* and *Mucuna pruriens*. *Biochem. Res Intern.* 2011:1-4.
 13. Lagnika L, Amoussa M, Adjovi Y, Sunni A. Antifungal, antibacterial and antioxidant properties of *Adansonia digitata* and *Vitex doniana* from Benin Pharmacopeia. *J Pharmacog and Phytother.* 2012;4(4):44-52.
 14. Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. *Bergey's manual of determinative bacteriology*, 9th ed. Baltimore and Wilkins 1994:783
 15. Moradian H, Bazargani A, Rafiee A, Nazarialam A. *In vitro* comparison of antimicrobial activity of aqueous decoction of *Coriandrum sativum* and Dentol Drop with chlorhexide on *Streptococcus mutans*. *Iranian Jour Microbiol.* 2013;5(3):239-243
 16. Irobi ON, Moo-Young M, Anderson WA, Daramola SO. Antimicrobial activity of bark extract of *Bridelia ferruginea* (Euphobiaceae). *J Ethnopharmacol.* 1994;4(3):185-190
 17. Akharaiyi FC, Oso BA, Boboye BE, Akinyosoye FA. Bacterial growth inhibition properties and in vivo antioxidant effect of *Spondias mombin* fruit juice. *Romanian Archiv of Microbiol and immunol.* 2017;76(1):27-35
 18. Pohl P, Dzimitrowicz A, Jedryczko D, Szymczycha-Madeja A, Welna M, Jamroz P. The determination of elements in herbal teas and medicinal plants formulations and their tisanes. *J. Pharm Niomed Anal.* 2016;S0731-7085(16):30042-5
 19. *Methods of Analysis of AOAC International* (18th ed.). Arlington VA; Association of Official Analytical Chemists 2005
 20. Jacop MB. *The chemical analysis of food and food products 3rd (Ed.)* Liston Education Publication Inc. *The Chemical Analysis of Food and Food Product.* 3rd(Ed) Liston Education Publication inc USA 1999:693-741.
 21. *Official Methods of Analysis of AOAC International* (20th eds). AOAC International, Maryland, USA. 2016
 22. *The British Pharmacopedia. Recommendation of Medicine* 1988;Vol I & II(1988).
 23. Kolawole SO, Kolawole OT, Akanji MA. Effects of aqueous extract of *Khaya senegalensis* stem bark on biochemical and hematological parameters in rats. *J Pharmacol Toxicol.* 2011;6(6):602-607
 24. Akharaiyi FC, Ezeanya-Bakpa CC, Otumala SO. Inhibition of methicillin resistant *Staphylococcus aureus* (MRSA) with underutilized medicinal plants extracts. *Int Medi Jour.* 2021;28(06):4953-4964
 25. Bouabdelli F, Djelloul A, Kaid-Omar Z, Semmoud A, Addou A. Antimicrobial activity of 22 plants used in urolithiasis medicine in Western Algeria. *Asian Pacific J Trop Dis.* 2012;S530- S535.
 26. Tavares LS, Silva CFS, Souza VC, Silva V, Diniz CG, Santos MO. Strategies and molecular tools to fight antimicrobial resistance. *Frontiers in Microbiol.* 2013;4:412
 27. Tiburski JH, Amanuri R, Rosires D, Ronoel L, de Oliveira G, Sidney P. Nutritional properties of yellow mombin (*Spondias mombin* L.) pulp. *Food Res Inter* 2011;44:2326-2331.
 28. Akharaiyi FC, Boboye B, Adetuyi FC. Antibacterial, phytochemical and antioxidant activities of the leaf extracts of *Gliricidia sepium* and *Spathodea campanulata*. *World Appl Sci Jour.* 2012;16(4):523-530
 29. Vunchi MA, Umar AN, King MA, Liman AA, Jeremiah G, Aigbe CO. Proximate, vitamins and mineral composition of *Vitex doniana* (black plum) fruit pulp. *Nigerian J Bas and Appl Sci.* 2011;19(1):97-101
 30. Aiwonegbe AE, Iyasele JU, Momodebe RO. Characterization of a natural dye produced from the alcoholic extract of African black plum (*Vitex doniana*) fruit pulp using wool fabric. *Proceedings of the 16th Annual International Conference of Nigeria Material Congress, Material Science and Technology Society of Nigeria (MSN).* 2017;105-109
 31. James D. Abu. Development of a sweetener from black plum (*Vitex doniana*) fruit. *Int J Food Proper.* 2002;5(1):153-159.