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PROXIMATE COMPOSITION, PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF THE METHANOL AND ACETONE EXTRACTS OF *VITEX DONIANA* FRUIT PULP

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ABSTRACT

The phytochemical constituents and the antimicrobial activity of the methanolic and acetone extracts of *Vitex doniana* (Sweet) fruit were investigated to ascertain its biological potentials. The study revealed that the fruit contained alkaloids, flavonoids, saponins, phenols, tannins, steroids and cardiac glycoside. The percentage proximate composition of the fruit of the plant were as follows: moisture 10.00%, ash 18.00%, crude fibre 4.50%, crude protein 0.60%, carbohydrate 43.20% and crude lipids 23.70%. The elemental analysis confirmed the presence of calcium, magnesium, potassium and sodium. The methanolic and acetone extracts of the fruit of the plant were tested against *Escherichia coli, Staphylococcus aureues, Pseudomonas areuginosa* and *Klebsiella pnuemoniae* by the agar (Mullen-Hinton) diffusion method. The zone of inhibition for the methanol extract ranged between 10.50 to 21.00 (mm) for all concentrations (1000, 500 and 250 mg/ml) . While the acetone extract only inhibited the growth of *Escherichia coli* to 7.50mm at a concentration of 1000mg/ml. The results obtained indicate that the fruit extracts have antimicrobial importance and can be utilized in the treatment of some ailments and malnutrition.

Key words: antimicrobial, extract, phytochemical, proximate, *Vitex doniana*

INTRODUCTION

The use of plants for the treatment of diseases and maintenance of good health has been well researched (Gill, 1992; Edeoga and Eriata, 2001; Moerman, 1996). Kambba and Hassan (2010) reported that plants and plant-based products are the bases of many modern pharmaceuticals used today for the treatment of various ailments. According to WHO (2001), about 80% of the world populations depend on plants based medicine for their health care. In WHO report (1996), it was also stated that the majority of the population in the developing countries (like Nigeria) still rely on herbal medicines to meet their health needs.

Modern society is now embracing the use of plants and plant-based products to meet societal health needs due to the fact that indiscriminate use of commercial antibiotics commonly utilized in the treatment of infectious diseases has led to the development of multiple drug resistance with attendant adverse effect on the host (Gupta *et al*., 2008). This emergence of pathogens resistant to antibiotics as a result of their excessive use in clinical and veterinary applications represents a serious public health concern (Keymanesh *et al*., 2009).

For more than three decades now, the world has experienced pathogenic-resistant bacteria which have been causing major health problems even though the pharmaceutical industries have produced large quantities of antibiotics. The resistance of bacteria and fungi to these drugs is becoming increasingly important (Lagnika *et al*., 2012). This resistance has led to the search for plants with antibacterial and antifungal activity in recent years. Other factors responsible for the use of plants in traditional as well as in modern medicine include safety, cost effectiveness (Koche *et al*., 2011) and adulteration of synthetic drugs (Shariff, 2001). Antimicrobials from plant origin have been found to have great therapeutic potentials (Werner *et al*., 1999). Such plants have been effectively used both in the treatment of infectious diseases to mitigated many of the side effects that are associated with synthetic antimicrobials (Perumalsamy and Ignacimuthu, 2000).

Black plum (*Vitex doniana* sweet) is of the family of verbanaceae. It is a tree crop that grows in open woodland and savannah regions of tropical Africa including northern, eastern and western Nigeria. (Dalziel and Hutchison, 1955). It produces fruits which are plum-like, sweet and edible. The fruit is

green when matured and changes to dark brown when fully ripe with the pulp surrounding a hard endocarp containing 1-4 seeds (Okigbo, 2001). In Nigeria it is known by the local names: *Dinyar* in Hausa; *Galbihi* in Fulani*; Ori nla* in Yoruba; *Ucha koro* in Igbo (Sofowora, 1993) and *Oriri* in Esan. A syrup similar to honey can be produced from the fruit and its physiochemical and sensory results show that it can be substituted for other syrup as nutritive sweetener (Egbekun *et al.*, 1996).

In ethnomedicine, the hot aqueous extract of *V. doniana* leaves is used for the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea and dysentery. The root has been used to treat epilepsy, nausea, and colic (Iwu, 1993). The stem bark extract of the tree is used for the control of hypertension, treatment of stomachache, pains, disorders, indigestion and sterility (Ladeji and Okoye, 1996). It has also been used for the production of dyestuff for textile materials (Tadzabia, *et al*, 2013 and Aiwonegbe, *et al*, 2017).

The current study was therefore undertaken to determine the antimicrobial activity of the phytochemicals in *Vitex doniana* fruit pulp and also to know its proximate and light metal compositions.

MATERIALS AND METHOD

Procurement and Preparation of Plant Material

The ripe fruits of *Vitex doniana* were obtained from the central market at Uromi, Edo State, Nigeria. The plant was identified by Dr. Emmanuel I. Aigbokhan of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria.

The fresh fruits were rinsed lightly with distilled water to remove sand and other debris. It was then manually milled through a stainless sieve of aperture or mesh size of 90 μm. The fruit pulp that passed through the sieve was collected in a container while the seed and the seed coat (pericarp) remained on top of the sieve. The fruit pulp was then spread on a flat pan previously coated with aluminum foil and allowed to undergo drying in a thermo-stated oven (*i-therm* AI-7741, LAB-TECH, INDIA) regulated at 60^0C. Drying is continued for 2 weeks until the fruit pulp hardens and moisture content reduced to a minimum. The dried fruit pulp is then ground into powder and stored in air-tight containers for analysis.

Phytochemical screening

The phytochemicals in *V. doniana* fruit pulp were qualitatively analyzed using the methods described by Harborne (1998) and Trease and Evans (2002).

Proximate Analysis

Proximate composition and light metal analysis were carried out using the methods described by Ikhuoria *et al,*. (2008).

Antimicrobial Activity and Minimum Inhibitory Concentration (MIC) Test

The sensitivity of the test organisms to the methanolic and acetone extracts of *V*itex *doniana* was carried out using the diffusion method described by Ebi and Ofoefule (1997) while the minimum inhibitory concentration was determined by the agar dilution method described by Baron and Finegold (1990). Both tests were carried out in the Laboratory of the Department of Microbiology, University of Benin, Benin City, Nigeria.

RESULTS AND DISCUSSION

The results for the phytochemical screening of *Vitex doniana* fruit pulp are presented in Table1 below. Alkaloids and flavonoids were present in minute quantities. Tannins and phenols were moderately available while cardiac glycosides, saponins, reducing sugars, steroids and terpenoids were abundantly present. The results compare favourably with those obtained for the leaves of *V. doniana* by Osuagwu and Eme (2013). Other researchers like Dauda *et al,*. (2011), Iwueke *et al,*. (2006) and Kubmarawa *et al,*. (2007) have also reported the presence of alkaloids, tannins, glycosides, steroids and flavonoids in the extracts of leaves, bark and stem of *V. doniana*.

Table 1: Phytochemical composition of *Vitex doniana* fruit pulp

Phytochemicals	Results
Phenols	++
Steroids	+++
Flavonoids	+
Alkaloids	+
Saponins	+++
Tannin	++
Reducing sugar	+++
Terpenoids	+++
Cardiac glycosides	+++

KEY: **(+)** = present; **(-)** = absent; **(+ +)** = moderately present
(+ + +) = abundantly present

The presence of these metabolites suggests great potentials for the fruit to be source of useful phytomedicines. The presence of flavonoid in fruits extracts indicates their possible application as anti-inflammatory and anti-allergy agents (Ishurd *et al.*, 2012). Flavonoids and phenols in the fruits also account for their antioxidant properties (Ogunleye and Ibitoye, 2003).

Presence of terpenoids indicates possible use of the fruits as anti-tumor and anti-viral agents as some terpenes are known to be cytotoxic to tumor cells (Kilani, 2006).

Fruits containing saponins are believed to have antioxidant, anticancer, anti-inflammatory, and antiviral properties (Philips, 2005). Saponins help humans to fight pathogenic microorganisms, increase the efficacy of certain vaccines and knock out some kinds of cancer cells, especially those of the blood and lungs. Saponins serve as innate antibiotics that help in fighting infections and microbial invasion of the body. These compounds are also capable of reducing cholesterol and bile acids level by forming complexes (Sheren, 2011). Saponins have also been found to be useful in the management of hypercholesterolaemia (Hostettmann and Martson, 2005).

Table 2: Proximate Composition of Vitex doniana **fruit pulp**

Parameters	Values (%)
Moisture content	10.00
Crude protein	0.60
Crude fat	23.70
Crude fibre	4.50
Ash content	18.00
Carbohydrate (NFE)	43.20

Table 2 above shows the result for the proximate composition of *Vitex doniana*. It has a high carbohydrate value of 43.20% which makes it an energy-giving fruit. It also has a considerable fat (lipid) content of 23.70%. It has a considerably low protein content of 0.60%. when compared to 10.0 % reported by Nnamani *et al.*, (2009). But Generally speaking, this low value for crude protein makes the fruit very suitable for consumption by aged people whose liver may not be able to digest high quantity of protein. Crude fibre content of 4.50% obtained from *V. doniana* fruit is low compared to the 15.0% reported for *V. doniana* leaves by Nnamani *et al.*, (2009). For a particular food sample, ash content gives a measure of the total mineral content. The fruiut pulp of V. *doniana* analyzed gave 18.00% as ash content. This is high compared to 5.27% reported

by Agbede and Ibitoye (2007). This difference may be as a result of the different prevailing environmental conditions in the places where the samples were cultivated.

It has been shown that though most fruits have high carbohydrate content, differences in values can however occur depending on the fruit type, maturity and environmental factors (Dreon *et al.,* 1990). The sample analysed gave a carbohydrate content of 43.20% which is higher than 28.40% obtained by Vunchi *et al.,* (2011). It is however low when compared to 67.0% reported in *V. doniana* leaves (Nnamani *et al*., 2009).

Table 3 below shows the mineral content of *Vitex doniana* fruit pulp. Potassium had the highest value (166.72 mg/100 g) while sodium had the lowest (27.37 mg/100 g). These values are considerably high when compared with those obtained by Bello, *et al*., 2014 as the macro-nutrient content of the leaves of *V. doniana*. They reported calcium, potassium and iron to be 3.36 g/100 g, 1.13 g/100 g and 0.12 mg/kg respectively but copper, chromium, nickel and zinc were found to be below detection limit.

The calcium in the fruits might be beneficial in preventing calcium deficiency related diseases like osteoporosis. Potassium plays an important role in controlling skeletal muscle contraction and nerve impulse transmission (Mensah *et al*., 2008).

Table 3: Mineral Content of *Vitex doniana* Fruit Pulp

Metal	Concentration (mg/100g)
Ca^{2+}	96.19
Mg^{2+}	58.35
K^{+}	166.72
Na^{+}	30.48

The fruit may therefore be recommended to patients with soft bone problems. Ladeji, *et al*., (2005) extracted and analyzed the bark of *Vitex doniana* and found it to contain much more potassium and phosphate than calcium, magnesium and zinc and iron. Therefore it was concluded that, the use of *Vitex doniana* to control postpartum bleeding may be justified.

Table 4(a): Zone of inhibition (in mm) of methanol extract of *Vitex doniana* fruit pulp

Bacterial isolates	Concentration				
	DMSO (1 ml)	Raw (mg/ml)	1000 (mg/ml)	500 (mg/ml)	250 (mg/ml)
Escherichia coli	0.00	19.00	18.00	16.80	14.10
Pseudomonas aeruginosa	0.00	16.00	12.00	11.00	10.50
Staphylococcus aureus	0.00	18.00	15.00	12.00	11.00
Klebsiella pnuemoniae	0.00	25.00	21.00	19.00	17.00

Table 4(b): Zone of inhibition (in mm) of acetone extract of *Vitex doniana* fruit pulp

Bacterial isolates	Concentration				
	DMSO (1 ml)	Raw (mg/ml)	1000 (mg/ml)	500 (mg/ml)	250 (mg/ml)
Escherichia coli	0.00	7.80	7.50	0.00	0.00
Pseudomonas aeruginosa	0.00	0.00	0.00	0.00	0.00
Staphylococcus aureus	0.00	0.00	0.00	0.00	0.00
Klebsiella pnuemoniae	0.00	0.00	0.00	0.00	0.00

Table 5: Antibiotic susceptibility testing (positive control)

Antibiotic	**Potency (µg/ml)**	Pseudomonas aeruginosa **(mm)**	Escherichia coli **(mm)**	Staphylococcus aureus **(mm)**	Klebsiella pneumonia **(mm)**
Perfloxacin	10.00	27.00	22.00	25.00	0.00
Streptomycin	30.00	19.00	0.00	19.00	0.00
Septrin	30.00	5.00	8.00	21.00	0.00
Gentamycin	10.00	15	18.00	16.00	0.00
Ciprofloxacin	10.00	25.00	21.00	14.00	0.00
Amoxicillin	30.00	23.00	11.00	16.00	0.00
Augmentin	30.00	4.50	12.00	18.00	0.00
Chloranphenicol	30.00	8.00	17.00	17.00	0.00
Tarivid	10.00	21.00	14.00	19.00	0.00

The results of the antimicrobial activity of *Vitex doniana* fuit pulp are shown in Tables 4a and 4b above. Also the result for the antibiotic susceptibility testing of the microbes is shown in Table 5. Generally, the methanol extract had a larger zone of inhibition and higher activity than the acetone extract. The methanol extract when compared with other antibiotics stands fairly well and so may be used as an alternative in the pharmaceutical industry for production of drugs for the treatment of infectious diseases. The methanol extract inhibited the growth of *E. coli, P. aeruginosa, S. aureus and K. pneumoniae* at all concentrations. It was most active against *K. pnuemoniae*. Earlier studies by NNMDA (2008) and Arbonnier (2004) have also revealed the microbial inhibitory effect of the leaves of *V. doniana*. Similarly, according to Latifou *et al.*, (2012) the methanol extract of the leaves of *Vitex doniana*, inhibited the growth of *E. coli* but *S. aureus* and *P. aeruginosa* were unaffected. Osuagwu and Eme (2013) also reported the ethanolic extract of *V. doniana* leaves to be active against *E. coli* (14.00 mm), *S. aureus (*11.00 mm), *S. typhi* (11.00 mm) and *P. aeruginosa* (5.70 mm). This suggests that the fruit may have more antibacterial property than the leaves.

CONCLUSION

The nutritional evaluation of *Vitex doniana* fruit pulp has shown that it contains significant levels of macro and micro nutrients i.e. carbohydrate, crude protein, crude fat, crude fibre, moisture, ash (minerals) and low content of anti-nutrients. Consumption of this fruit is therefore encouraged for maintenance of good health and vitality. The low moisture content suggests that the fruit can be stored (fresh) for more than 24 hours without deterioration.

This study has also showed that extracts of *V. doniana* fruit have antimicrobial activity on some pathogens. This implies that it has medicinal value. Therefore, it could be exploited for use in the formulation of cheap alternative antimicrobial drugs to cure or control human infectious diseases. Conclusively, the cultivation of *Vitex doniana* (Black plum/*Oriri*) should be encouraged being a plant with appreciable nutritional potentials and possible economic and health benefits.

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