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COMPARATIVE STUDY ON THE PROXIMATE, MINERAL, PHYTOCHEMICALS AND ANTIMICROBIAL ANALYSIS OF *Daniellia oliveri, Leptadenia hastata* AND *Vitex doniana* LEAVES

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ABSTRACT
Plant biodiversity contribute significantly to human health. Plants also serve as curing agents since long and also provide basic nutrients like carbohydrate, protein, fibre etc. These nutrients have essential role in supporting human body requirements. The phytochemical constituents and the antimicrobial activity of Daniellia oliveri, Leptadenia hastata and Vitex doniana were investigated to ascertain its biological potentials. The study revealed that Leptadenia hastata contain significantly ($p < 0.05$) higher moisture content (17.61 ± 0.02%), ash (1.51 ± 0.01%) and crude fibre (2.60 ± 0.09%) compared to the respective values of (10.87 ± 0.02%, 2.42 ± 0.03%), (1.04 ± 0.01%, 1.00 ± 0.00%) and (2.49 ± 0.01%, 2.42 ± 0.03%) for Vitex doniana and Daniellia oliveri. While Daniellia oliveri contain significantly ($p < 0.05$) higher crude protein (25.03 ± 0.26%) and fat content (6.86 ± 0.04%) compared to (17.96 ± 0.07%, 7.85 ± 0.05%) and (5.42 ± 0.02%, 4.31 ± 0.01%) for Leptadenia hastata and Vitex doniana. On the other hand, the carbohydrate content was significantly ($p < 0.05$) higher for Vitex doniana (73.44 ± 0.01%) than 62.27 ± 0.37% for Daniellia oliveri and 54.88 ± 0.27% for Leptadenia hastata. The calculated energy (4.1094kcal/100g) for Daniellia oliveri was significantly ($p < 0.05$) higher compared to (3.6395kcal/100g), (3.4014kcal/100g) for Vitex Doniana and Leptadenia hastata leaves. Mineral elements concentrations were low in all the three samples except for phosphorous. This study revealed that the three samples contained flavonoids, saponins, alkaloids, carbohydrate, cardiac glycoside, phenols and tannins. The result of the antimicrobial analysis reveals that all the samples were very effective against S. aureus with MIC ranging from 0.4-0.8 µg/ml and also against K. pneumoniae with MIC ranging from 0.6-1.4 µg/ml. The results obtained indicates that these leaves have antimicrobial importance and can be utilized in the treatment of some ailments and malnutrition.
Keys words: Leaves, Medicinal plants, Microbial load, Minerals, Phytochemical

INTRODUCTION

Plants play a very important role in our lives. Human use an extensive variety of plant derivatives as food, drugs and nutritional increments (Sen and Samanta, 2014). The use of medicinal plants has attained a commanding role in health systems all over the world, this involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions.

Even today, plants are not only indispensable in health care, but form the best hope of source for safe future medicines. Though recent researches on herbal plants or medicine, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine. Medicinal plants contain a wide variety of secondary metabolites or compounds such as tannins terpeniods, alkaloids, flavonoids that dictates the therapeutic potency of plants most especially the microbial activities (Evans *et al.,* 2002).

Plants possessed basic nutrients like fats, protein, carbohydrates etc. In the development of human culture, medicinal plant have played an essential role, for example religious and different ceremonies (Hosseinzadeh *et al.,* 2015).

Daniellia oliveri is a species of tree in the family Fabaceae. It is native to tropical West and Central Africa, while in Nigeria it is traditionally known as 'Maje' in Hausa language, 'Emi iya' in Yoruba and 'iya /ozabwa /agba' in Igbo language. The tree can grow up to 18 – 24 in height, the leaves are with a glabrous common stalk of 0.15 – 0.46m long swollen base, 4 – 9 pairs of leaflets 0.06 – 0.08m. The leaves are aphrodisiac, astringent and diuretic. Dry leaves powder is also administered to treat yellow fever, backache and headache and for wound healing (Ahmadu *et al.,* 2007).

Vitex doniana, commonly known as black plum, is a deciduous flowering tree growing up to 20m in height in tropical Africa. It is a tree crop that grows in open woodland and savannah regions of tropical Africa. 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; *Dinya* in Hausa, *Galbihi* in Fulani, *Ori nla* in Yoruba, *Ucha koro* in Igbo. In ethnomedicine, the hot aqueous extract of *Vitex doniana* leaves is used for the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhea and dysentery. The root has been used to treat epilepsy, nausea and colic The stem bark extract of the tree is used for the control of hypertension, treatment of stomach ache, pains, disorders, indigestion and sterility. It has also been used for the production of dyestuff for textile materials (Aiwonegbe *et al*., 2018).).

Leptadenia hastata is a perennial liana species of the Apocynaceae family. *Leptadenia hastata* is an important emergent local food of Africa. It is among the few vegetables present in almost all seasons, on dry lands and in sandy soil. Through this it provides food security. It is usually sown near houses so that it is available for human consumption. Many studies have revealed the potential of *L. hastata* for different uses (Saiba et *al.,* 2016). *Leptadenia hastata* is often used traditionally for hypertension, catarrh, skin diseases, wound healing, prostrate complaints and as an aphrodisiac. *Leptadenia hastata* is reported to contain alkaloids, saponins, phenolic glycosides, tannins, flavonoids and triterpenes (Thomas, 2012).

MATERIALS AND METHODS

All the equipment used were calibrated: Moisture balance (Model: MB200, England OHAUS), Analytical weighing balance (OHAUS Analytical plus, England), Atomic Absorption Spectroscopy (GBC AvantaGF300, Switzerland), Micro-kjedahl apparatus (S. W. Germany), Muffle furnance (Korl- kolb, Germany). All the reagents used in this study were of analytical grades purity; glass-wares of appropriate sizes were properly washed using appropriate solvents and rinsed with deionized water.

Sample Collection

Fresh leaves of *Daniellia oliveri, Leptadenia hastata and Vitex doniana* were collected from Chaza, Suleja Local Government, Niger State, Nigeria. The samples were identified and authenticated by a taxonomist at the Herbarium Unit, National Institute for Pharmaceutical Research and Development, Idu- Abuja, Nigeria. A voucher specimen was deposited at the herbarium of the institute with voucher specimen numbers NIPRD/H/7272, NIPRD/H/7273, NIPRD/H/7274 for *Daniellia oliveri, Leptadenia hastata and Vitex doniana* respectively. The leaves were washed and air dried. The leaves were pounded into powder form using mortar and pestle. Then powdered samples were stored in an air tight container, properly labelled and kept at room temperature for subsequent analysis.

Proximate Analysis

Standard methods (AOAC, 2006) were employed for the proximate analysis. The moisture content was determined by drying two grams of the samples in an oven at 105^0 C to constant weight. The ash content was determined by the incineration of 2g sample in a muffle furnance at 550^0 C for 3 hours. Crude lipid was exhaustively soxhlet extracted from 2g sample with n-hexane for 8 hours. The nitrogen (N) content was estimated by micro-kjedahl method and crude protein (CP) content calculated as N% $\times$ 6.25. Crude fibre content was determined by treating 2g sample with 1.25% (W/V) H_2SO_4 and1.25% (W/V) NaOH. The available carbohydrate (CHO) was calculated by difference Calorific value (CV) was determined using the following equation.

$CV(kcal/100g) = (CHO \times 4) + (CL \times 9) + (CP \times 4)$

Mineral Analysis

Mineral analysis was carried out after digestion using the double acid digestion method (James, 1995). P, K, Na, Ca and Mg were determined by atomic absorption spectrophotometric (AAS) method.

Phytochemical Screening

The phytochemical screening was carried out according to the methods outlined in (Kumbhar and Godghate, 2015).

Antimicrobial Activity and Minimum Inhibitory Concentration (MIC) Test

Test Organisms

Pure isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Enterococus faecalis* and *Candida albicans*, collected and biochemically confirmed at the Central Service Laboratory, National Cereals Research Institute Badeggi, Niger State, Nigeria, were used for this study.

Inoculum Preparation

A loopful of the test organism (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Enterococus faecalis* and *Candida albicans*) was taken from their respective agar slants, sub-cultured into 5 mL of nutrient broth and incubated at 37°C. Following incubation at 37°C for 24 hrs, organisms were diluted with normal saline to a turbidity that was equivalent to 0.5 Mc Farland standard (10^6 CFU)/mL) (Olawepo *et al.,* 2014).

Antimicrobial Susceptibility Test

The antimicrobial susceptibility study of the samples was carried out by colourimetric assay of broth micro dilution technique as described by [CLSI, 2012]. About 50 µL of Mueller Hinton (MHB) broth was dispensed into sterile wells of 96 microwell plate from row 2-12. 100 µl of each sample was transferred into well 1 of the microwell plate in duplicate. About 50 µL of the solution in well 1 was transferred to well 2, mixed thoroughly and repeated through to well 11 where 50 µL was discarded. The wells (1-12) were inoculated with 50 µl of the diluted microorganisms and incubated at 37^0C for 24 hours. After incubation period, the wells were added 25 µL of 3-(4, 5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium (Sigma Aldrich), re-incubated over-night and observed for absence or presence of microbial growth by colour change in the wells. The MIC was defined as the lowest drug/extract concentration that prevented the colour change of the dye to pink. Colourless well was interpreted as there is no microbial growth and pink color was interpreted as growth occurrence.

Statistical Analysis

All the data obtained were subjected to statistical analysis using PRISM Graphad Software version 5. One-way analysis of variance (ANOVA) was performed on the data where Mean, range. Standard deviation, standard error and p-value were obtained. The level of statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The basic nutritional importance of plants is assessed by their content of protein, carbohydrate, fats and oils, minerals, vitamins and water which are responsible for the growth and development in man and animals (Akinniyi and Waziri, 2011), they are the essential nutrients of life. Their quality and quantity makes them basic factors and important for the selection of plants for nutritive value, systematic classification and plant improvement programs (Nisar *et al.,* 2009). Proximate composition of plants provides a valuable information about its medicinal and nutritional quality. The values of moisture, ash, fats, protein and carbohydrate of the samples are presented in Table 1. The present study showed that *Leptadenia hastata* leaves has significantly ($p < 0.05$) higher moisture content than *Vitex doniana* leaves and *Daniella oliveria* leaves. However, the study was similar with that reported by (Hussain *et al.,* 2010) on medicinal plants, which indicated that leafy vegetables have higher moisture content compared to non- leafy plants. Moisture content depends on the environmental conditions such as humidity, temperature, harvest time and climate as well as storage conditions (Hussain *et al.,* 2011). It is also reported that moisture content varied in different species which is independent on the physiology and environment of the plant (Tijjani *et al.,* 2016).

Ash content of *L. hastata* leaves was also significantly ($p < 0.05$) higher than *V. doniana* and *D. oliveria.* The results obtained in this study ash were lower than that reported by (Aiwonegbe *et al.*, 2018), Tijjani *et al.,* 2016, Olanipekun *et al.,* 2016). This difference may be as a result of the different prevailing environmental conditions in the places where the samples were cultivated. The ash content is a reflection of the mineral contents preserved in that plant. Many medicinal plants like *Urginea altissina* possesses low ash content (Hussain *et al.,* 2011).

Table 1: Proximate composition of the samples (% dry matter)

Parameter	DO	LH	VD
Moisture	2.42 ± 0.03^{a}	17.61 ± 0.02^{c}	10.87 ± 0.02^{b}
Ash	1.00 ± 0.00^{a}	1.51 ± 0.01^{c}	1.04 ± 0.01^{b}
Crude lipid	6.86 ± 0.04^{c}	5.42 ± 0.02^{b}	4.31 ± 0.01^{a}
Crude protein	25.03 ± 0.26^{c}	17.96 ± 0.07^{b}	7.85 ± 0.05^{a}
Crude fibre	2.42 ± 0.03^{a}	2.60 ± 0.09^{c}	2.49 ± 0.01^{b}
Carbohydrate	62.27 ± 0.37^{b}	54.88 ± 0.27^{a}	73.44 ± 0.01^{c}
Energy value	4.1094kcal/100g^{c}	3. 4014kcal/100g^{a}	3.6395kcal/100g^{b}

All values were mean ± standard deviation of three triplicate determinations.
Values in the same row with different superscript differs significantly (P < 0.05)
DO= *Daniella oliveria*, LH= *Leptadenia hastata*, VD= *Vitex Doniana*

The fat content of *D. oliveria* leaves was significantly (p < 0.05) higher with a value of 6.86 ± 0.04% than *L. hastata* 5.42 ± 0.02%, followed by *V. doniana*. 4.31 ± 0.01%. However, the fat content of *D. oliveria* is higher than that reported by (Olanipekun *et al.,* 2016). The results for *V. doniana* in this study is lower than that reported by Ibrahim *et al.,* 2012). The result obtained for *L. hastata* is also lower compared to that reported by (Uusiku *et al.,* 2010).

Percentage protein content of *D. oliveria* is significantly (p < 0.05) higher than *L. hastata* and *V. doniana*. The protein content of the three samples are comparable to that of other leafy vegetables where the protein content range from 1-7% of fresh weight or 8 to 30% of dry weight basis (Uusiku *et al.,* 2010). This study shows that the protein content of the samples is high and are within the recommended nutrient intake (Alexopoulou *et al.,* 2013) and are capable of providing the body with the essential amino acids required for growth (Igwenyi *et al.,* 2014).

L. hastata contain significantly (p < 0.05) high amount of crude fibre compared to *V. doniana*. and *D. oliveria.* These values are low when compared with that reported by (Hasan *et al.,* 2011). Carbohydrates have a vital role in living organisms. They can easily be oxidized to yield instant energy, their polymer act as energy storage molecules and their derivatives are found in a number of biological molecules including coenzymes and the nuclei aids (Hasan *et al.,* 2011). The carbohydrate composition of *V. doniana* leaves was significantly (p < 0.05) higher with a percentage of 73.44 ± 0.01%, D. *oliveria* have 62.27 ± 0.37% and *L. hastata have* 54.88 ± 0.27%. The result obtained in this study for carbohydrate percentage content which is high and this in comparison with the previous results, where carbohydrate content of leaves of *Bambusa vulgaris, Euphorbia hirta, lawsonia inarmic, Mimosa pudica* and *Persia americanas* were analyzed and all the plants had high carbohydrate content. It has been shown that though most plants have high carbohydrate content, differences in values can however occur depending on the plant, maturity and environmental factors (Abidemi *et al.,* 2013). Leaves of *D. oliveria* have higher energy value than *L. hastata* and *V. doniana.*

The result of mineral content of *D. oliveria, L. hastata* and *V. doniana* is presented in Table 2.

Phosphorous had the highest value in all the three samples analyzed. The sample *D. oliveria* contained significantly (p < 0.05) higher amount of (P) than *L. hastata* and *V. doniana.* These values are considerably high when compared with obtained by (Agbebe and Ibitoye, 2007). The amount of Mg in *L. hastata* was found to be significantly (p < 0.05) higher than *V. doniana* and *D. oliveria,* these values are considered very low compared to that reported by (Agbebe and Ibitoye, 2007).

Table 2: Mean Concentration of Mineral Content (µg/g) of the samples

Metal	DO	LH	VD
Phosphorus	27.11 ± 0.28^{a}	25.59 ± 0.08^{b}	24.26 ± 0.05^{c}
Potassium	2.13 ± 0.03^{c}	3.43 ± 0.02^{a}	2.99 ± 0.02^{b}
Sodium	1.05 ± 0.03^{b}	2.42 ± 0.02^{a}	2.42 ± 0.02^{a}
Calcium	2.50 ± 0.02^{a}	2.42 ± 0.02^{b}	2.42 ± 0.02^{b}
Magnesium	5.30 ± 0.02^{c}	6.41 ± 0.01^{a}	6.20 ± 0.02^{b}

All values were mean ± standard deviation of three triplicate determinations.
Values in the same row with different superscript differs significantly (P < 0.05)
DO= *Daniella oliveria*, LH= *Leptadenia hastata*, VD= *Vitex Doniana*

Leptadenia hastata contained **s**ignificantly ($P < 0.05$) high amount of K than *Daniella oliveria* and *Vitex Doniana* while Na and Ca were not significant ($P < 0.05$) different between *Leptadenia hastata* and *Vitex Doniana.* The three samples contained low amount of K, Na and Ca. From this study, the samples, by and large contained low amount of mineral elements. This is an indication that the samples are not good source of other macronutrients. The concentration of microelements was also low but compared favourably when their recommended dietary allowances were considered.

The phytochemical characteristic of *Daniella oliveria*, *Leptadenia hastata* and *Vitex doniana* is presented in Table 3 below. This study reveals that carbohydrate, flavonoids, alkaloids, tannins, saponins, cardiac glycoside, phenols and tannins were detected in all in samples. The results compare favorably with those obtained for the leaves of *Centtella asiatica*, *Daniella oliveria, Morinda citrifolia* and *Vitex doniana* by (Alagbe and Oluwafemi, 2019). The presence of flavonoids in the samples is an indication that the samples possesses antibacterial, antifungal and antioxidant properties (Krishnaiah *et al.,* 2009). Alkaloids have been suggested to be involved in antimicrobial, analgesics and antiplasmodic (Ishurd *et al.*, 2004). Plants containing saponins are believed to have antiviral properties (Iwu, 2004). The presence of these metabolites in these samples suggests great potentials for the leaves to be source of useful phytomedicines.

Table 3: Phytochemical screening of the samples

Phytochemicals	Test	Inference		
		DO	LH	VD
	Molish's test	+	+	+
Carbohydrate	Fehling's A and B	+	+	+
Flavonoids	Shinoda test	+	-	+
	Alkaline reagent	+	-	+
	Lead acetate	+	+	+
Saponins	Froth test	+	+	+
	Fehlings test	+	-	-
Phenols &Tannins	Ferric chloride test	+	+	+
Alkaloids	Mayer reagent	+	+	+
	Dragendroff's	-	+	-
	Wagner	-	+	-
	Hager test	-	+	-
Terpenes &sterols	Salkowski's test	-	-	_
Cardiac Glycoside	Keller killiani test	+	+	+

Keys: + = Presence; - = Absence.

Table 4: Result of Antimicrobial Minimum Inhibitory Concentration (µg/ml)

Sample Description	Method of extraction	*Stapylococcus aureus*	*E. feacalis*	*E. coli*	*K-Pneumonie*	*P-aeroginosa*	*Candida albicans*	*S-aureus*
VD	H_2O	0.6	7.6	4.2	0.8	2.4	6.6	4.2
DO	H_2O	0.8	5.8	1.6	0.6	2.6	5.4	0.8
LH	H_2O	0.4	4.3	3.4	1.4	3.8	4.3	1.6

The results of the antimicrobial activity of the samples are shown in Table 4. All the samples showed varying degrees of antimicrobial activity against the test organisms used. The minimum inhibitory concentration (MIC) of the samples used in this study were within the ranges of 0.4 - 7.6 µg/ml. *Vitex doniana* was able to inhibit the growth of the test organisms used in this study with the most effective against *Staphylococcus aureus* with MIC of 0.6 µg/ml, followed by *Klebsiella pneumoniae* with MIC of 0.8 µg/ml, *Vitex doniana* was also active against *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Enterococus faecalis* with MICs of 2.4, 4.2, 6.6 and 7.6 µg/ml respectively but not as effective as *S. aureus* and *K. pneumoniae*. *Daniella oliveria* also inhibited the growth of the test organisms with the most active against *K. pneumoniae* with MIC of 0.6 µg/ml and also very active against *S. aureus* with MIC of 0.8 µg/ml and the sample was also effective against *E. coli*, *P. aeruginosa*, *C. albicans* and *E. faecalis* with MICs of 1.6, 2.6, 5.4 and 5.8 µg/ml respectively but not as effective as *K. pneumoniae* and *S. aureus*. Finally, sample HH inhibited the growth of the microorganisms used with the most effective against *S. aureus* at concentration of 0.4 µg/ml

and also *K. pneumonia* with MIC of 1.4 µg/ml. *Leptadenia hastata* was also effective against *E. coli*, *P. aeruginosa*, *C. albicans* and *E. faecalis* with MICs ranging from 3.4 - 4.3 µg/ml respectively. It can be deduced from this result that all the samples were very effective against *S. aureus* with MIC ranging from 0.4 - 0.8 µg/ml and also against *K. pneumoniae* with MIC ranging from 0.6 -1.4 µg/ml.

CONCLUSION

The nutritional evaluation of *Daniella oliveria, Leptadenia hastata* and *Vitex doniana* leaves has shown that it contains significant levels of macro and micro nutrients (i.e. carbohydrate, crude protein, crude fat, crude fibre ash and moisture) while all the samples contain low content of minerals except phosphorous and magnesium. The samples have antimicrobial activity on some pathogen. This study has also showed that carbohydrate, flavonoids, alkaloids, tannins, saponins, cardiac glycoside, phenols and tannins were detected in all in samples. This implies that all the samples have medicinal value, consumption of these leaves is therefore encouraged for maintenance of good health and vitality.

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