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Research Article

AN INVESTIGATION OF THE PHYTOCHEMICAL AND ELEMENTAL CONTENT OF STEM BARK OF *VITEX DONIANA* SWEET (BLACK PLUM)

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ABSTRACT
Phytochemical and elemental contents of stem bark of V. *doniana* were investigated. The stem bark of V.*doniana* (5kg) was macerated with ethanol and thereafter sequentially extracted using chloroform, ethyl acetate and n-butanol. Moisture content and ash content were determined. Phytochemical analysis of the air dried matter, ethanol, chloroform, ethyl acetate and n-butanol of the stem bark extracts of V. *doniana* were carried out using standard procedures. The stem bark of V. *doniana* was ashed, digested and analysed for heavy and trace elemental content using atomic absorption and flame emission spectroscopy (AAS and EES). The percentage moisture content and ash value were 7% and 3%.The extracts concentrate yields of ethanol, chloroform, ethylacetate and n-butanol were 37%w/w, 1.25%w/w, 2.4%w/w, 3.36%w/w. Preliminary phytochemical screening revealed the presence of tannins, saponins, steroid, terpenes, phloba-tannins, carbohydrate, flavonoids and cardioative-glycosides in the dry matter, ethanolic, ethylacetate and n-butanol fractions.The chloroform fraction has only cardioactive glycosides and carbohydrate. Alkaloids and anthraquinone were absent in all the extracts. Results of elemental contents determination indicates that the concentration in ug/g of some trace essential elements such as Mangnese (Mn), Magnesium (Mg), Potassium (K) and Calcium (Ca) in the stem bark of *vitex doniana* to be within safety limits reported by World Health Organization. However the concentration of Potasium (K) is slightly above the WHO recommended level. Non-essential and toxic metals such as lead (Pb) and cadmium (Cd) were not detected while Iron (Fe), Zinc (Zn), Cobalt (Co), Sodium (Na), Copper (Cu) and Phosphrus (P) are found in low concentration. These essential elements detected in the stem bark of V. *doniana* are very much required by our bodies for biological and physiological functions that are necessary for maintenance of good health while the phytochemicals reported are implicated in having many pharmacological properties. Further research is going on purification, characterization, structural elucidation and assaying some of the pharmacological activities.

Key Words: *Phytochemical, Vitex doniana, Elemental*

INTRODUCTION
Nearly all cultures of the world, both ancient and the recent have heavily depended on plants as a therapeutic agents used in various forms. Plants play a major role in the treatment of diseases and still remain the foremost alternative for a large majority of people. (Adiaratou et al., 2005). The knowledge of plants if wisely utilized could draw out promising herbal leads. The World Health Organization (WHO, 1996) has reported that about 80% of the world population relies on traditional medicine to cure ailments (Farnsworth et al., 1985 and Harsha et al., 2002). Medicinal plants is defined as any plant in which one or more of its organs contain substances that can be used for therapeutic purpose and which are precursors for the synthesis of new drug (Sofowora,1993). Phytochemicals are rich source of organic substances which are used for production of many drugs. Investigation into therapeutic efficacy of plants is therefore urgently needed because of widely usage, acceptability and so many claimed activities for treatment of ailments and diseases. The phytochemicals that have medicinal importance are the secondary metabolites.

These secondary metabolites includes phenolic compounds such as tannins and flavonoids, glycosides such as cardiac, flavonoids, steroidal and saponins. Terpenes, alkaloids, volatile oil, gums and mucilage. These organic compounds were extracted, isolated, purified and subjected to detailed investigation for their medicinal/pharmacological properties by scientist using standard procedures so as to prove the claim therapeutic properties. Elemental research has definitely been part of this explosion of scientific knowledge (Said et al., 1996). Several studies have reported elemental contents in plant extracts which are consumed as herbal health drink or in orthodox medicine (Rajua et al., 2006).The qualitative estimation of various trace element concentration is important for determining the effectiveness of medicinal plants in treating various diseases and also to understand the pharmacological action (Rajua et al., 2006). Trace elements that are necessary for human health include Iron, iodine, copper, manganese, zinc, molybdenum, selenium and chromium etc (Hendler and Shelder, 1990). Herbs are not only provide us with chemicals of medicinal value but also provide us nutritional and trace elements (Zafar et al., 2010.)

Vitex doniana Sweet, a plant commonly known black plum, in English, Prunier noir in French, dinya in Hausa , ucha koro in Igbo, oori-nla in yoruba and ngarmi in Kanuri is a medium-sized deciduous tree, 8-18 m high, with a heavy rounded crown and a clear bole up to 5 m. V. *doniana* is from *Verbenaceae* family and abundantly occurring in savannah regions. It is a deciduous forest tree of coastal woodland, riverine and lowland extending as high as upland grassland, secondary forests, and dry forests. It can be found throughout tropical Africa. (Han-Jurgen, 1990; Hutchinson and Dalziel, 1964; Keay, 1964 and Kokwaro, 1976). Studies have reported the elemental and phytochemistry of root bark and leaves of V. *doniana*, however there is a scanty report on the stem bark. The objective of this research is to investigate the ash, moisture, phytochemical and elemental contents of stem bark of V. *doniana*.

MATERIALS AND METHODS

Sample collection and identification

The stem bark of V*itex doniana* leaves were collected in Kawuri village of Konduga (11°39′6″N 13°25′10″E) Local Government Area of Borno state of Nigeria. The plant specimen was identified and authenticated by a Plant Taxonomist, Prof. S.S. Sanusi of the Department of Biological Sciences, Faculty of Science, University of Maiduguri. The herbarium specimen with a voucher number 555C was deposited at the Post Graduate Research Laboratory, Department of Chemistry. The stem bark of the *Vitex doniana* was cleaned and air-dried in the laboratory. Five kilogram (5kg) of the stem bark of *Vitex doniana* was pulverised into a coarse powder using mortar and pestle.

Estimation of moisture content

Five grammes (5g) of V. *doniana* stem bark were placed in a weighed watch glass and dried in the oven at 80^{o}C to constant weight using standard procedures with constant checking for 2 hours. The percentage moisture content was then determined (AOAC,1984 and Radojevic and Baskin, 1999).

Ash value determination

Five grammes (5g) of V. *doniana* stem bark were put in a weighted acid washed and air dried porcelain crucible in a hot spot furnace, and the temperature was raised slowly until it reached 500^{o}C. it was then left there for about 4 hours. The crucible was removed from the furnace after ashing with a pair of thongs and weighted. The percentage ash value was then determined. (AOAC,1984, Radojevic and Baskin, 1999).

Extraction of stem bark

The weighed powdered air-dried sample (5kg) was macerated with ethanol for five days and partitioned sequentially with choroloform, ethyl acetate, and n-butanol. The extract obtained was concentrated to dryness in vacuo at 40°C using a rotary evaporator. The extract concentrate was labeled and the percentage yields were calculated in w/w.

Phytochemical analysis

The air died matter, crude extracts of the choroloform, ethyl acetate, n-butanol and ethanol were subjected to qualitative chemical screening for identification of the primary and secondary metabolites such as flavonoids, alkaloids, sterols, triterpenes, saponins, anthracenosides, tannins, polyuronides, emodol, etc as described by (Ioan, 1982; Sofowora,1993 and Trease and Evans 2002).

Elemental Contents Evaluation

The air dried sample (10g) was dried to a constant weigh in an oven at 80^{o}C . The sample (5g) was then ashed in a hotspot furnace at 500^{o}C for 3 hours. The ashed material was then digested using standard procedures (Radojevie and Baskin 1999) and analysed for the determination of trace and heavy elements present in *Vitex doniana* stem bark using a combination of flame emission spectrometry (FES) (GallenKamp) and Atomic Absorption Spectrometry (AAS) (SPG Unicam Model No.1) at appropriate wavelength, temperature and lamp current for each element under study (Radojevie and Baskin 1999). Flame emission spectrometry (FES) (Gallen Kamp FGA 330) was use to determine sodium and potassium. Elements such as magnesium, calcium, iron, lead zinc, manganese, cadmium and copper was determined by atomic absorption spectrometry (AAS).

RESULTS AND DISCUSSION

Moisture Content and Ash Value

The percentage moisture content and ash value are 7% and 3%. The total ash could serve as a standard in determining the amount of residual substances not volatilized when the drug is ignited (African Pharmacopoeia, 1986).

The extracts concentrate yields of ethanol, chloroform, ethylacetate and n-butanol were 37 %w/w, 1.25%w/w, 2.4%w/w and 3.70%. As seen from table 2 and 3, the results of the pre-liminary phytochemical analysis revealed the presence of tannins, phlobatannins, saponins, carbohydrates, cardioactive glycoside, flavonoids, steroids and terpene in the dry matter, ethanolic, ethylacetate and n-butanol fractions. The chloroform fraction has only cardioactive glycosides and carbohydrates. Alkaloids and anthracenosides were conspicuously absent in all the extracts. Plants are rich bio resources of drugs. A number of interesting outcomes have been found with the use of a mixture of -natural products or plant extracts to treat diseases (Gibbons, 2003). Phytochemical studies are important aspects of medicinal plants research and are used in evaluating the presence of the chemical constituents in plants (Abdurahaman, 2004). The phytochemicals that have medicinal importance are the secondary metabolites. These secondary metabolites includes phenolic compounds such as tannins and flavonoids, glycosides, cardioactive glycoside, steroidal glycosides and saponins. Terpenes, alkaloids, volatile oil etc. Tannin have astringent properties which are important in wound healing (Pondromoli and Grazi, 1969). Flavonoid compounds are is implicated in having an antipyretic property, anti-inflammatory and anti-oxidant property. (Trease and Evans, 2002). Saponins have an expectorant action that is very useful in management of upper respiratory tract. Cardioactive glycosides are naturally cardioactive drugs used in treatment of congestive heart failure and cardiac arrhythmia (Brian et al.,1985)

Table 1. Percentage yield, colour and texture of EtOH, $CHCl_3$, EtOAc and n-butanol extracts of stem bark of V. *doniana*

Extract fraction	%yield(w/w)	Colour	Texture
EtOH	37%	black	gummy
$CHCl_3$	1.25%	dark brown	gummy
EtOAc	2.40%	light brown	gummy
n-butanol	3.70%	dark brown	powder

Table 2. Phytochemistry of the dry matter

S/N	Class of Chemical Components	Results
1.	**Test of Alkaloids**	
	Dragendoff reagent	-
	Mayers reagent	-
2.	**Test for Flavonoid**	
	Shinoda Test	-
	Lead acetate test	++
	Sodium hydroxide test	++
	Ferric Chlorides test	+
3.	**Test for carbohydrate**	
a.	General Test (Molish test)	+
b.	Test of monosaccharide	-
c.	Test for reducing sugar (Fehling test)	++
d.	Combine reducing sugar test	++
e.	Test for ketoses	+
f.	Test for pentose	+
4.	**Test for tannins**	
	Ferric chloride	+
	Lead acetate	+
	Hydrochloric acid test	-
5.	**Test for free Anthraquinones (Bontrase)**	-
	Test for combined anthraquinone	-
6.	**Test for cardio active glycoside**	
	Salkowski test	+
	Liebermann Burchard test	+
7.	**Terpenoid test**	+
8.	**Test for soluble starch**	+
9.	**Test for phlobatannins**	-
10.	**Test for saponins**	
i.	Frothing test	+
ii.	Fehling test	++

Key - *Absent*
\+ *Present in low concentration*
++ *Present in moderate concentration*

Table 3. Phytochemistry of ethanolic (EtOH), chloroform ($CHCl_3$) ethylacetate (EtOAc), and n-butanol extracts.

S/N	Class of Chemical Components	EtOH	$CHCl_3$	EtOAc	n- butanol
1.	**Test of Alkaloids**				
	Dragendoff reagent	-	-	-	-
	Mayers reagent	-	-	-	-
2.	**Test for Flavonoid**				
	Shinoda Test	-	-	+	+
	Lead acetate test	++	-	++	++
	Sodium hydroxide test	++	-	+	+
	Ferric Chlorides test	++	-	+++	++
3.	**Test for carbohydrate**				
	General Test (Molish test)	+	-	+	++
	Test of monosaccharide(Barfoed test)	-	-	-	-
	Test for reducing sugar (Fehling test)	++	-	-	++
	Combine reducing sugar test	++	-	-	++
	Test for ketoses	+	-	-	+
	Test for pentose	-	-	-	+
4.	**Test for tannins**				
	Ferric chloride	++	-	++	++
	Lead acetate	++	-	++	++
	Hydrochloric acid test	-	-	-	-
5.	**Test for free Anthraquinones (Bontrase)**	-	-	-	-
6.	**Test for combine anthraquinone**	-	-	-	-
7.	**Test for cardio-active glycoside**				
	Salkowski test	++	++	+	++
	Liebermann Burchard test	++	++	+	+
8.	**Terpenoid test**	+	++	++	++
9.	**Test for soluble starch**	+	+	+	+
10.	**Test for phlobatannins**	+	-	+	+
11.	**Test for saponins**				
	Frothing test	++	-	++	++
	Fehling test	++	-	+	++

Key - *Absent*
\+ *Present in low concentration*
++ *Present in moderate concentration*

The elemental analysis results in figure 1 and appendix I indicates that the concentration of some trace essential elements such as Mn, Mg, K and Ca in the Stem bark of *vitex doniana* appeared to be within safety limits reported by World Health Organization (1996). However the concentration of K is slightly

Appendix 1. Concentrations of trace and heavy elements in digested sample of stem bark of *Vitex doniana*

ELEMENTS	Concentration (ug/g)	WHO Standard (1999) (ug/g)
Potasium (K	121	10-100
Sodium (Na)	163	400-500
Mangnesium (Mg)	103	100-200
Iron (Fe)	8.4	50-5000
Zinc (Zn)	6.0	150-20000
Copper (Cu)	13.8	100-300
Calcium (Ca)	5423	3600-80000
Suphur (S)	63	8-500
Cobalt (Co)	7.8	100
Lead (Pb)	ND	5-30
Phosphorus (P)	22	100-200
Mangnese (Mn)	560	100-20000

above the WHO recommended level. Trace elements are chemical elements required by our bodies for biological and physiological functions that are necessary for maintenance of good health. The elements

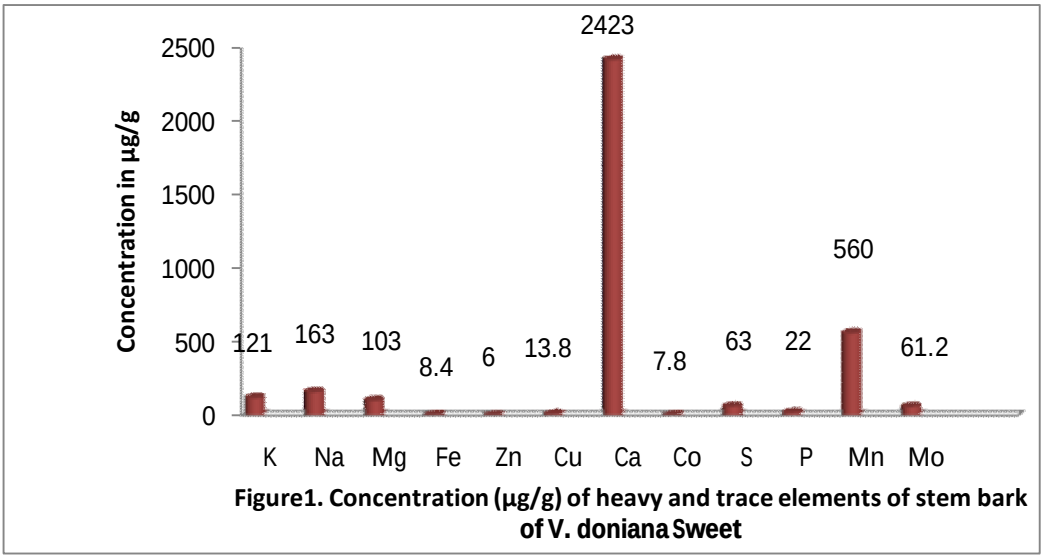

Figure1. Concentration (µg/g) of heavy and trace elements of stem bark of V. doniana Sweet

present in the plant may be an indication of the types of minerals presents in the soil. Mineral elements are essential in many vital processes in both plants and animals. According to Kaneez et al., (2003), elements such as Mg in plants lowers the cholesterol level and alleviate heart diseases, Fe deficiency is associated with myocardial infection while Zn is important in wound healing. Mn is essential for normal functioning of central nervous system and is good anti-oxidant (Bibi et al., 2006) . Ca is needed in the development of bone teeth, regulates heart rhythm, helps in blood clotting, maintain proper nerve and muscle functions and lowers the blood pressure (Badahur et al., 2011). Non-essential and toxic metals

such as Pb and Cd were not detected in the stem bark *Vitex doniana*. Fe, Zn, Co, Na, Cu and P are found in low concentration. Further research are going on in purification, characterization and structural elucidation of active component in the stem bark of V. *doniana* and some pharmacological studies.

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