PHYTOCHEMICAL ANALYSIS, SOME ELEMENTAL AND VITAMINS COMPOSITION OF BLOOD PLUM (*ANACARDIACEAE*)

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ABSTRACT

This research was undertaken to carry out the phytochemical analysis, determine some elements and vitamins composition of the stem bark extract of blood plum. All the analyses in this research were done using standard procedures. Standard methods were employed for all the studies. The result showed the presence of terpenoid, saponin, flavonoid and phlobatannin. The result also showed the presence of Na, Mn, Mg, Fe, Cu, Pb, Zn and Ca. From the result, Fe has the highest concentration (16.21 ± 2.5×10-3 mg/Kg) with Cu having the least concentration (1.07± 1.35×10^{-4}mg/Kg).The result revealed the presence of some essential vitamins (A, B1, B2, K, and C). Vitamin A was found to be the highest (365.20 ± $1.23\times10^{-}$4 IU/100g), with B2 the lowest (0.14 ± 1.35×10^{-4} mg/100g). Vitamin C was found to have the concentration of (10.40± 2.74×10^{-4}mg/100g).From this study, it can be concluded that the stem bark extract of blood plum is a potential blood tonic and it also have an antioxidant potential due the presence of Vitamin C and flavonoids which may play a significant role in treatment of oxidative stress , cardiovascular arrests, inflammation, cancer and diarrhoea

KEYWORDS: Blood Plum, Elements, Vitamins C, Stem bark Extract and phytochemicals

INTRODUCTION

Traditionally the use of plants parts as source of herbal preparation for treatment of various ailments are based on experience passed from generation to generation (Mensah and Okoli, 2009). The herbal knowledge or practices known by traditional healers are jealously guarded with utmost secrecy for economic reasons. According to Obute (2007), many traditional herbal practitioners also tend to hide the identity of plants used for different ailments largely for fear of lack of patronage should the patient learn to cure himself. Thus to mystify their trade, cultivation of the plant is not encouraged, consequently collection is virtually from the wild.

A number of medicinal plants abound in Nigeria Flora (Gbile, 1986) which is the richest country in West Africa with regards to medicinal plants resources. The country exhibits a wide range in terms of climate and topology which has a bearing on its vegetation and floristic composition. Some records on herbs that are used to manage common ailments in Nigeria are found in the work of Adegoke *et al.*,(1968), Obute (2007), Okoli *et al.,* (2007), Mensah *et al.,* (2009) among others. Herbal preparations are used in traditional medicine as crude drugs in various dosage forms as whole, crushed, powdered forms, decoctions, dried extracts, infusions, poultices and tinctures (Awosika, 1993). Many of these plants however have been investigated in recent times and found to contain active substances that are medically useful, where as many more are yet to be scientifically investigated.

A number of plants extracts used by natives in various parts of the world as blood additives include: pistachio found in United State, South West Asia and Mediterranean region, wood sorrel, Butterbur, found in Europe, and Asia, Blood plum found in Nigeria, Ghana, Cameroon, and other part of Africa (Arbonnier , 2004).

This present study is on Blood plum (*Anacardiaceae*). The plant is widely distributed in Nigeria, among which are Mubi in Adamawa state, Taraba, Nassarawa, Bauchi, Jos and others. It is a tree which can grow up to 8m high by 65cm girth, of rocky situations of the dry savannah from Upper Volta to Nigeria, and in Cameroon, Sudan, and Ghana. Burkill (1985). The bark contains a clear gum, the fruit is a red purple drupe, and nearly 2.5cm long like the temperate plum. The pulp is thin edible with an acid but resinous taste. The kernels are somewhat oily and are also edible (Bussan 1965, Dalziel 1937). It is called “Jan danyaa” “Dan danya” or “tsamiyar Lamaruuddu”= tamarind of the giant in Hausa, “tursuhi’ or “Tursahi” (plural “Tursuuje”) in Fulfulde, “Turzin”, “Trezein” in Fali, “Trezah’ in Gude, “Mbaumae” in Bachama and Michika people call it “Drne”. This plant is used by many herbalists as medicine: for the remedy of blood deficiency, for barred women to bear children, to regain appetite (oral interview). A bark-infusion is taken by Fulani of Northern Nigeria for “sawoora” (hepatitis), (Irvine 1961). In Nasarawa a decoction along with Natron (“2Kanwa”) is taken for sleeping sickness (Irvine 1961).

The aim of this study is to investigate some elements and vitamins that are associated with red blood cell production, to see if

the stem bark extract could serve as an alternative blood tonic.

MATERIALS AND METHODS

Sample collection and preparation

The stem bark was collected at Bahuli Mubi North Local Government Area of Adamawa State. It was identified and authenticated by a botanist in the Department of Biological Sciences of the Adamawa State University, Mubi.

The stem bark of the plant was harvested and dried under shade; the dried stem bark was grounded into fine powder using pestle and mortar, and kept in a polythene bag before the onset of the experiment.

Extraction Technique

Aqueous Extraction Method: - following the procedure of (Sofowora 1995, Onyeyili 2001). 20g of the powdered sample was accurately weighed into a beaker and 200 mL of distilled water was added to the sample in the beaker, it was allowed to boil for 2 hours by heating it up to 200˚C to remove all water soluble compounds from the sample. This was allowed to cool, after cooling the extract was filtered using filter paper.

Phytochemical Analysis.

The aqueous extract of the plant was subjected to qualitative chemical screening for the identification of Saponin, Tannin, Phlobatannin, Alkaloid, Flavonoid and Terpinoid. Following the procedure described by Sofowora (2008) and modified by Toma *et al.*, (2011).

Test for Saponin.

2g of the powdered sample was boiled in 20 mL of distilled water in water bath and filtered. 10 mL of the filtrate was mixed with 3 drops of olive oil and shaken vigorously, formation of emulsion indicate positive result.

Test for Alkaloids.

The aqueous extract (0.2mL) was stirred and placed in 1% aqueous hydrochloric acid (5mL) on a steam bath. Then 1 mL of the filtrate was treated with Mayer´s reagent (3 drops) while another portion was similarly treated with Dragendorff´s reagents. Turbidity or precipitation with these reagents was considered as evidence for the presence of alkaloid.

Test for Flavonoids.

2 mL of the portion of the aqueous extract was heated, and a metallic magnesium and concentrated hydrochloric acid (5 drops) was added a red or orange coloration indicates the presence of flavonoid.

Test for Terpenoid

The aqueous extraction 5 mL was mixed with 2 mL of chloroform and 3 mL of concentrated H_2SO_4 was added to form a layer. A reddish brown coloration of the interface formed indicates
positive result.

Elemental Analysis

Determination of Na, Ca, Zn, Mg, Fe, Cu, Pb and Mn with atomic absorption spectrometer was as described by (Toma *et al., 2011*). About 1.35g dried sample was transferred to the destruction tube (kjedal flask), 25 mL HNO_3 was added, three boiling chips added and a funnel was placed on top of the destruction tube. The tube was heated to 100˚C, 125˚C and 150˚C and maintained at each interval for 1hour, 15minutes and 10minutes respectively.

The tube was heated to 200˚C and HNO_3 was added. The mixture was concentrated to about 5 mL. After cooling, 1 mL 30% H_2O_2 was destructed for 10minutes, it was cooled again after adding 3 mL 30% H_2O_2 and was heated, destructed for 10minutes 25 mL water was added and heated till boiling. The whole sample was cooled and filled up to the mark, mixed and was allowed to settle for at least 6hours. The absorbance of the clear supernatant was measured and the various concentrations was determined from the standard calibration curve.

Determintion of Vitamin A, B1, B2, C and K by Isocratic High Performance Liquid Chromatography (HPLC)

Buffer preparation

0.9411g of sodium hexane sulphonic acid was weight into 1litre volumetric flask, 750 mL of deionised water to dissolve, 10 mL glacial acetic acid was also added and was made up to mark with deionised water.

Sample Preparation

2 mL of aqueous sample was put into 25 mL standard volumetric flask and was made up to mark with buffer/deionised water/diluents. It was shaked centrifuged, decanted and was filtered using HPLC grade filter paper.

Procedure:

The manufacturer's manual was followed to condition the HPLC system. The standard was injected with a 100 IU syringe and was loaded, and the chromatography data was recorded, and calibration curve was prepared. The sample was also injected and loaded and the samples chromatography was recorded using the calibration curve. The amount of vitamins in the sample was quantified.

RESULTS AND DISCUSSION

The result of phytochemical screen is shown in Table 1. The result showed that terpenoid, saponin, flavonoid and phlobatannin are present, while alkaloid and tannin are below detectable level.

The result of elemental analysis of the plant extract is shown in Table 2. From the result Fe has the highest concentration ($16.21\pm 2.43\times10^{-3}$ mg/Kg) with Cu having the least concentration ($1.07\pm1.35\times10^{-4}$ mg/Kg), other element present are Pb ($5.00\pm1.34\times10^{-4}$ mg/Kg), Ca ($14.67\pm1.36\times10^{-4}$ mg/Kg), Na ($5.50\pm1.35\times10^{-4}$ mg/Kg), Mg ($2.45\pm 2.43\times10^{-3}$ mg/Kg), Mn ($5.20\pm1.35\times10^{-3}$ mg/Kg) and Zn ($3.00\pm 4.09\times10^{-4}$ mg/Kg).

Table 3 shows the result of vitamin composition of the plant extract. The result showed the presence of vitamins, A, B_1, B_2, C and K, from the result it is shown that blood plum has high amount of vitamin A ($365.20\pm1.23\times10^{-4}$ IU/g) followed by vitamin C ($10.40\pm 2.74\times10^{-4}$ mg/g), vitamin K ($6.85\pm 2.86\times10^{-4}$ mcg/g), vitamin B_1 ($0.417 \pm 1.35\times10^{-4}$ mg/g), and vitamin B_2 ($0.140 \pm1.35\times10^{-4}$ mg/g) has the least concentration.

Table 1: Result of qualitative Phytochemical Analysis

Blood Plum (stem bark extract)	
Terpenoid	+
Saponin	+
Alkaloid	_
Phlobatannin	+
Flavonoid	+
Tannin	_

+ = present - = Absent

Table 2: Result of the elemental analysis

Elements	Blood plum (mg/Kg)
Zn	$3.00 \pm 4.09\times10^{-4}$
Cu	$1.07 \pm 1.35\times10^{-4}$
Mn	$5.20 \pm 1.35\times10^{-3}$
Mg	$2.45 \pm 2.86\times10^{-3}$
Fe	$16.21 \pm 2.45\times10^{-3}$
Ca	$14.67 \pm 1.36\times10^{-4}$
Pb	$5.00 \pm 1.35\times10^{-4}$
Na	$5.50 \pm 1.35\times10^{-4}$

All values represent Mean ± Standard error of mean (SEM); n=3

Table 3: Results of the vitamins composition

Vitamins	Blood plum
A (IU/100g)	$365.20 \pm1.23\times10^{-4}$
B1 (mg/100g)	$0.42 \pm 1.35\times10^{-4}$
B2 (mg/100g)	$0.14 \pm 1.35\times10^{-4}$
C (mg/100g)	$10.40 \pm 2.74\times10^{-4}$
K (mcg/100g)	$6.85 \pm 2.85\times10^{-4}$

All value represent Mean ± Standard Error of Mean (SEM); n=3

DISCUSSION:

The result of phytochemical screening of the stem bark extract of blood plum showed that terpenoid, saponin, phlobatannin, flavonoid are present, and were known to show medicinal activities as reported by (Francis *et al.,* 2002).

The presence of saponin and phlobatannin are important interest for pharmaceuticals due the uses of saponin as adjuvant in vaccines and phlobatannin as sex hormone as reported by Toma *et al.,* (2009). The presence of terpenoid and flavonoid indicate that the plant may be used as natural flavour additive for food, aromatherapy and as anticancer and antidiarrheal activities as also reported by Toma *et al.*, (2009) and Glenn (2010). That could also contribute to the non-offensive odour of the decoction as claimed by the traditional users of this plant extract.

The result of elemental analysis of the stem bark extract of the plant shows the presence of Na, Pb, Zn, Fe, Mn, Mg, Ca and Cu. Fe has the highest concentration ($16.21\pm 2.43\times10^{-3}$ mg/Kg), with Cu having the least concentration ($1.07\pm1.35\times10^{-4}$ mg/Kg).

The presence of Na indicates that the plant can regulate blood pressure and blood volume, and also good for muscle and nerve functions (Lichtenstein 2006).

The presence of Ca and Mg indicate that the plant is good for vascular contraction and vasodilation, muscle function, nerve transmission and hormonal secretion (Washington 2010).

The presence of Fe shows that the plant is essential for red blood cell production and oxygen transport in the body (Andrews 1999).

The presence of Cu, Mn and Zn indicate that the plant is essential for immune function, protein synthesis, blood clotting factors, sex hormones, formation of haemoglobin and for secretion and potentiating insulin action, this has been also reported by (Mild 1996, Toma *et al.*, 2011 and University of Mary Land medical centre, (2011).

The presence of Pb in the stem bark extract of the plant which is below the toxic level when compared to the normal Pb level of 10mcg/g in children and 40mcg/g in adults (Toma *et al.*, 2011). This shows that the plant may only be toxic when taken in high amount for a very long period of time.

The high concentration of Fe and the presence of Na, Mg, Mn, Zn, Ca and Cu show that the plant extract is a very good blood tonic.

The results of vitamin composition of the stem bark of blood plum indicate the presence of thiamine (B1), Riboflavin (B2), Retinol (A), Ascorbic acid (C), and Mephyton (K). The presence of thiamine and riboflavin though not in a significant amount is an indication that the plant can help in body metabolism of carbohydrates, protein, fats and also helps to strengthen the immune system and improves the body's ability to withstand stressful conditions University of Mary Land medical centre, 2011). The presence of vitamin C shows that the plant is a natural anti-oxidant, and can help the body to be resistant against infection agents, counter inflammation and scavenge harmful free radicals (Toma *et al.,* 2009). The presence of vitamin A also shows that the plant is essential for vision and for maintaining healthy mucus membrane and skin. The presence of vitamin K shows that the plant is essential for many clothing factors in the blood as well as in bone metabolism and helps reduces Alzheimer's disease in the elderly. www.nutrient-and-you.com 2009-11).

The presence of vitamin B1, B2 and C show that the plant can boast blood if taken in reasonable amount

The study on phytochemical screening and some elemental and vitamin composition of the stem bark extract of blood plum showed the presence of saponin terpenoid, flavonoid, phlobatannin, the extract is found to be rich Fe, Ca, Na, Mg, , Zn, Cu and vitamin A, B1, B2, C and K. The high concentration of Fe, the presence of other elements like Na, Mg and the presence of vitamins B1 and B2 indicate that the plant is a good source as a blood tonic, good to regulate blood pressure, good for muscle and nerve function. It also have an antioxidant potential due the presence of Vitamin C and flavonoids which may play a significant role in treatment of oxidative stress , cardiovascular arrests, inflammation, cancer and diarrhoea.

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