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Effects of Ethanol Leaf Extract and Fractions of *Bixa Orellana* L. On Haematological Profile in Albino Rats

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ABSTRACT

The study investigated the effects of leaves of *Bixa orellana* L. on some haematological and biochemical indices of albino rats. The dried leaf powder was subjected to extraction with ethanol and the resultant extract further underwent fractionation with n-hexane, chloroform, ethyl-acetate and butanol to yield their respective fractions and the aqueous extract. Preliminary phytochemical screening of the leaf and analysis of the effects of the extracts and fractions on haematological parameters were carried out. Their effect(s) on serum electrolyte concentrations of sodium (Na), potassium (K), bicarbonate (HCO_3) and chloride ions (Cl^-) was also determined. Median lethal dose (LD_{50}) value of the ethanol leaf extract of *Bixa orellana* L. was determined in two phases using 45 mice, administered between 10 – 3000mg/kg of the extract. Forty-five albino rats were randomly divided into nine groups of five rats each. Group I served as the control and received 5ml/kg of distilled water, while groups II, III and IV were orally administered with 169.71mg/kg, 339.4mg/kg and 509.1mg/kg of the ethanol extract respectively; the remaining five groups were administered 339.4mg/kg of the butanol, ethyl-acetate, chloroform, n-hexane and aqueous fractions of *Bixa orellana* for 21 days. Results of the phytochemical screening of *Bixa orellana* revealed the presence of flavonoids, tannins, terpenes, saponnins and cardiac glycosides. The LD_{50} value of *Bixa orellana* when administered intraperitoneally was 1697.06 mg/kg. Ethanol extract of *Bixa orellana* significantly (P=0.05) decreased the WBC particularly neutrophils, PLT and Na concentration in a dose-dependent manner. It also significantly (P=0.05) increased in a dose-related fashion the RBC, PCV, Hb and K. All the fractions significantly (P=0.05) decreased the PLT and the WBC with the ethyl-acetate fraction showing the most anti-platelet effect. The ethyl-acetate, chloroform and n-hexane fractions significantly (P=0.05) increased the RBC, PCV and Hb. The high dose of ethanol extract, butanol and ethyl-acetate fractions showed a significant (P=0.05) reduction of Na while K was increased significantly (P=0.05) by the low and middle dose of ethanol extract, and n-hexane fraction. These results indicate that *Bixa orellana* leaf increases RBC, PCV and Hb, decreases WBC and has an anti-platelet effect. The results lend scientific support to some folkloric uses of *Bixa orellana* leaves in traditional medicine.

Keywords: anti-platelet, *Bixa orellana*, ethanol, extract, haematology and phytochemical.

I. INTRODUCTION

It is generally known that the consumption of a variety of local herbs and vegetables by man contributes significantly to the improvement of human health, in terms of prevention and/or cure of diseases because plants have long served as useful and natural sources of therapeutic agents (Chevellier,1996). The medicinal application of plants is as old as human existence itself (Schultes and Raffauf, 1990). World Health Organisation's estimation of 1996 stated that about four billion people, that is to say 80% of the world's population, apply plants in medicine (WHO, 1996). Medicinal plant has been defined as any part of the plant in which one or more of its organs contain substance(s) that can be used as a cure to ailment or which are precursors for the synthesis of useful drugs (Sofowora, 1993). Traditional medicine has been brought into focus for meeting the goal of a wider coverage of health care delivery, not only in Africa, but also to a large extent in

all countries of the world (Elujoba *et al*., 2005). Currently, there is an on-going world-wide green revolution which is mainly premised on the belief that herbal remedies are safe and less damaging to the human body than synthetic drugs.

Bixa orellana L. popularly known as Lipstick tree or Annato, is a profusely fruiting shrub or bushy tree with glossy ovate leaves and fruits of bright red to brownish colour. It is known as "*Nsaneto*" in the Ibibio-speaking region of Akwa Ibom State (Etukudo, 2003). Many medicinal values are attributed to the leaves of *Bixa orellana*. The genus name of *Bixa orellana* is said to be probably derived from the Portuguese word "*Biche*" meaning "*beak*" which refers to the beak-shaped seedpods, while the species name is given in memory of Francisco de Orellana, a Spanish conquistador of the 16th century, who accidentally discovered the Amazon.

Bixa orellana is a profusely fruiting shrub or bushy tree which ranges from 300-1000 cm in height. Its glossy, ovate leaves are evergreen with reddish veins. They have a round, cordate and cuspidate tip. The flowers are pink, white, or some combination, and are 4 – 6cm in diameter. From the flower protrudes a striking two-valve fruit, covered either with dense soft bristles or a smooth surface. These round fruits, approximately 4cm wide, appear in a variety of colours; scarlet, yellow, brownish-green, maroon, and most commonly bright red. When ripe, they split open to reveal numerous amount of small, triangular, fleshy seeds approximately 50 in number, about 5mm in diameter and covered with red-orange pulp, the embryo of which is poisonous (Chopra, 1949).

The leaves of *Bixa orellana* possess many medicinal properties and have been used in traditional medicinal practices of many countries all over the world. The leaves are believed to have astringent, antiseptic, emollient, antibacterial, antirust, expectorant, febrifuge, stomachic, purgative, anti-inflammatory, hypoglycaemic and anti-dysenteric properties. Infusions of the leaves are taken as tea for their diuretic properties and as anti-gonorrheal treatment. The seed and leaves are used to prepare remedies for a variety of illnesses such as tonsillitis, asthma, pleurisy, rectal disorders, headache, jaundice, sunstroke and burns (De-Oliviera *et al.*, 2003; Etukudo, 2003). In Nigeria the whole seeds of *Bixa orellana* is crushed and the powder used in food preparations to enhance food colour and as a body dye (Etukudo, 2003). The bark of *B. orellana* has been shown to elicit protective activity through anti-oxidant activity on acetaminophen-induced hepatic damage in rats. Blood is the life-maintaining fluid that circulates through the body. It is important for pulmonary and tissue respiration, as a medium of endocrine and neuro-humoral transmissions, biotransformation and metabolic excretion, nutritional and immunological processes, as well as homeostatic responses (Adebayo *et al*., 2005). Blood is a good indicator to determine the health of an individual and a pathological reflector of the whole body (Joshi *et al*., 2002). The biochemistry of the blood is directly linked to the functional capacity of the blood, and the functional capacity is associated with the status of the blood components. This research is aimed not solely on evaluation of an acclaimed therapeutic benefit but towards evaluation of the effects of leaf of *Bixa orellana* in the blood and its components; towards a potent but safe application of the leaf in herbal medicine, provision of insight into other beneficial potentials of *Bixa* and discovery of a potent novel anti-platelet/anticoagulant drug.

Figure 1. Plate 1 : Plant showing leaves and fruits of *Bixa orellana* L.

II. METHODS AND MATERIAL

A. *Plant Materials/Identification*

Fresh leaves of *Bixa orellana* were collected from the wild in Ikot Akpan, Afaha Obio Eno, Ibiono Ibom Local Government Area of Akwa Ibom State, Nigeria on

September 6th, 2012 by the corresponding author. The plant identification was done by Dr. (Mrs.) M. E Bassey, a plant taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The vouchers specimens were deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.

B. *Preparation of Plant Extract*

The leaves were washed, shade–dried and extracted with 70% ethanol (v/v) by cold extraction for 72 hours. The extracts were evaporated to dryness at 40^{o}C in a water bath. The semi-solid extract was stored in the refrigerator at -4^{0}C for further use.

C. *Phytochemical Screening*

The experiment was carried out in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo. The phytochemical screening involved the simple chemical test to detect the presence of secondary metabolites. Standard methods of Trease and Evans (2009) were used for phytochemical screening.

D. *Preparation of Plant Extract*

The leaves *Bixa orellana* were washed, shade–dried, pulverised to powder. The total weight after powdering was 1.2kg. 950g of the coarse powdered leaves macerated with 1200ml of 70% ethanol in a conical flask for 72 hours. The extracts were evaporated to dryness at 40^{o}C in a water bath. The semi-solid extract was stored in the refrigerator at -4^{0}C for further use. The solution was successively fractionated in a separating funnel with n-hexane, chloroform, ethyl-acetate and finally butanol. Fractionation of the ethanol extract yielded 12.8 g of n-hexane fraction, 6.25 g of chloroform fraction, 10.54 g of ethylacetate fraction, 4.32 g of butanol fraction and 3.20 g of the aqueous fraction.

E. *Determination of Median Lethal Dose (LD_{50}) Value*

Median Lethal Dose (LD_{50}) value of the ethanol extracts was determined using the modified method of Lorke (1983) as described by Pawar *et al.* (2006). Swiss albino mice (*Mus musculus*) were used for the study. The evaluation of the acute toxicity was done in two phases and the extract administered intraperitoneally. In phase one, three groups of five mice each were treated with 10 mg/kg, 100 mg/kg and 1000 mg/kg doses of the ethanol extract respectively. The control group received normal saline. The mice were observed for physical signs of toxicity and mortality such as writhing, tail licking, palpitations, staggering etc. within 24 hours.

Based on the result from phase one, thirty fresh mice were weighed and grouped into six groups of five mice each. They were administered with different doses of the extract (1000-5000mg/kg).

They were observed for physical signs of toxicity and mortality for 24 hours. The LD_{50} was calculated as geometric mean of the maximum dose producing 0% mortality (D_0) and the minimum dose producing 100% mortality (D_{100}).

$$LD_{50} = \sqrt{D0} \times D100$$

F. *Experimental Design*

The forty-five rats were randomly assigned into nine groups consisting of five animals in each group. The ten percent (10%), twenty percent (20%) and thirty percent (30%) of LD_{50} were used as working doses (low, middle and high dose respectively).

Group I received 5ml/kg of normal saline, group II received 169.7mg/kg, group III received 339.4mg/kg and group IV received 509.1mg/kg of ethanol extract of *Bixa orellana* leaf.

After fractionation of the ethanol extract of *Bixa orellana* leaf, group V received 339.4 mg/kg of the aqueous fraction, group VI received 339.4 mg/kg of the butanol fraction, group VII received 339.4 mg/kg of the ethyl-acetate fraction, group VIII received 339.4 mg/kg of the chloroform fraction and group IX received 339.4 mg/kg of the n-hexane fraction of *Bixa orellana* leaf. The daily oral administration lasted for twenty one days (21-days) and during this period animals were allowed free access to feed and water *ad libitum*.

G. *Collection of Blood Samples*

After 21 days of daily oral administration, additional two days were allowed for complete metabolism and absorption of the drugs. Forty eight hours after the last administration, the animals were anaesthetised with chloroform, dissected to exposed the cardiac cavity of the heart, blood was obtained using a sterile syringe (5ml) by cardiac puncture and carefully discharged into plain sample bottles and ethylene diamine tetraacetic acid (EDTA) sample bottles respectively, for serum electrolyte and haematological analysis. The sample bottles were labelled accordingly. The blood in the plain sample bottles were allowed about one hour to clot before separating the serum from the clot by centrifugation. The serum obtained was used for analysis of serum electrolyte concentration.

H. *Determination of Haematological Indices*

The determination of the haematological indices was done within two hours of sample collection using Mindary 5 Path Differential BC 5300 Automated Hematology Analyser in the Haematology Unit of the University of Uyo Teaching Hospital. The methods of Alexander and Grifiths (1993 a, b) and Lewis *et al*. (2001) was adopted.

I. *Serum Electrolyte Analysis*

The blood samples for serum electrolyte analysis were centrifuged after one hour of blood collection using a centrifuge at the Department of Pharmacology and Toxicology Laboratory, University of Uyo, Uyo to separate the serum from other blood components. The serum was frozen in plain sample bottles and transferred to Enquiries Medical Laboratory in Uyo, Akwa Ibom State. The concentration of sodium (Na), potassium (K), bicarbonate (HCO_3) and chloride (Cl^-) ions of blood samples were determined by flame photometry, volumetric analysis, and titrimetric method of Schales and Schales as described by Varley *et al*. (1980), Kulpmann (1992) and AOAC (2003).

J. *Statistical Analysis*

This was carried out using windows Statistical Package for Social Sciences (SPSS) version 17.0. One way analysis of variance was adopted for comparison, and the results were subject to post hoc test using least significance difference (LSD). The data expressed as mean ± standard error (P=0.05) were considered significant.

III. RESULT AND DISCUSSION

The phytochemical screening of the leaf of *Bixa orellana* revealed the presence of flavonoids, tannins, terpenes, saponins, and cardiac glycosides (Table 1). The mice were administered intraperitoneally with a single dose of 10-1000 mg/kg in phase 1 and 1200-3000mg/kg of *Bixa orellana* leaf extract after being starved for 18 hours. Intraperitoneal route was chosen because of its sensitivity and rapid results. *Bixa orellana* leaf extract produced various degree of toxicity, ranging from decreased respiration to mortality from dose 1400 mg/kg and above. The intensities of these effects were proportional to the dose administered. The intraperitoneal LD_{50} of *Bixa orellana* was 1697.06mg/kg

The results of the effects of oral administration of *Bixa orellana* ethanol leaves extract and butanol, ethyl-acetate, chloroform, n-hexane and aqueous fractions for 21-days caused a significant (P=0.05) decrease in the WBC and absolute neutrophils count. All the fractions and the different doses of the ethanol extract significantly (P=0.05) decreased the PLT with the ethyl-acetate fraction showing the most anti-platelet effect. There was a significant decrease (P=0.05) in P-LCR with most fractions and with the highest dose of ethanol extract. There was no significant change in the PDW. The ethanol extract also significantly (P=0.05) increased the RBC, PCV, Hb, and MCV. Likewise, the ethyl-acetate, chloroform and n-hexane fractions significantly (P=0.05) increased the RBC, PCV and Hb. These results indicate that *Bixa orellana* leaf increases RBC, PCV and Hb, decreases WBC and has anti-platelet effect (Tables 2-7).

The results of electrolyte concentration obtained from the experiments showed a marked significant (P=0.05) decrease in Na in the groups that received the highest dose of ethanol extract, the butanol and ethylacetate fractions when compared to the control group. Significant (P=0.05) increase in K was also recorded for the low and high doses of the ethanol extract, and the chloroform and n-hexane fraction (Tables 8 and 9).

Table 1: Result of the phytochemical screening of *Bixa orellana* L. leaves.

Test	Observation	Inference
1. Alkaloids		
(i) Dragendorff's reagent	No turbidity, No colour change, No reaction	-
(ii) Mayer's reagent	No turbidity, No colour change, No reaction	-
(iii) Picric acid test	No turbidity, No colour change, No reaction	-
(iv) Wagner's reagent	No colour change, No reaction	-
2. Saponins		
(i) Foamtest	Formation of emulsion, milky colouration	+++
(ii) Froth test	Honeycomb froth	++
(iii) Sodium bicarbonate test	Brown coloured ppt.	++
3. Flavonoids		
(i) Shinoda'stest	Red colour observed	+++
(ii) Ammonia test	Formation of yellow spot on filter paper	+++
(iii) Sodium hydroxide test	Yellow colour turn creamy on dilute SO_4	+++
4. Tannins		
(i) Ferricchloridetest	Blue-green ppt. observed	+++
(ii) Bromine water test	Adding bromine water the solution decolourised	++
5. Cardiac glycosides		
(i) Salkowski test	Reddish-brown ring at interface	++
(ii) Lieberman's test	Green ring at interface	++
(iii) Keller-kilian's test	Brown ring at interface	++
8. Anthraquinones		
(i) Free anthraquinone	No colour change, No reaction	-
(ii) Combined anthraquinone	No colour change, No reaction	-

Legend: = Absent, + =Trace, ++ = Moderate, +++ = Abundance

Table 2 : Effect of 21 days oral administration of ethanol extract of *Bixa orellana* leaves on haematological indices in albino rats.

Group	Treatment (mg/kg)	RBC ($x10^{12}$/l)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
I	Control (distilled water)	6.30± 0.10	11.63± 0.19	37.50± 0.36	59.40± 0.40	18.43± 0.77	31.00± 0.18
II	169.7 (low dose)	6.60± 0.15*	11.93± 0.89*	40.36± 2.40	50.80± 0.63	17.53± 0.63	28.87± 0.14
III	339.4 (Middle dose)	6.79± 0.28*	12.00± 0.10*	41.30± 2.26	61.17± 0.25*	18.00± 0.74	29.37± 0.63
IV	509.1(High dose)	6.83± 0.10*	12.63± 0.28*	42.87± 1.0*	62.73± 0.80*	18.50± 0.55	29.50± 0.60

Results were expressed as mean values ± standard error of mean. *P=0.05 when compared with the control group. Sample size (n=5). Hb= haemoglobin concentration, RBC= red blood cell,
PCV= Packed cell volume, MCV=mean corpuscular volume, MCH= mean corpuscular haemoglobin and MCHC= mean corpuscular haemoglobin concentration

Table 2: Effect of 21 days oral administration of fractions of *Bixa orellana* leaves on haematological indices in albino rats.

Group	Treatment (mg/kg)	RBC ($x10^{12}$/l)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
I	339.4 (aqueous)	6.41± 0.13	11.70± 0.27*	39.10± 0.90	58.90± 0.20	18.53± 0.62	29.78± 0.10
II	339.4 (butanol)	5.83± 0.23*	10.23± 0.11	33.10± 0.45	56.30± 0.30	17.63± 0.18	31.47± 0.52
III	339.4 (ethyl-acetate)	6.43± 0.14*	12.31± 0.60*	39.53± 0.60	58.16± 0.25	18.53± 0.40	30.33± 0.10
IV	339.4 (chloroform)	6.87± 0.18*	12.76± 0.14*	41.62± 0.39*	58.00± 0.15	18.97± 0.35	31.07± 0.58

Table 4 : Effect of 21 days oral administration of ethanol extract of *Bixa orellana* leaves on haematological parameters in albino rats.

Group	Treatment (mg/kg)	WBC ($x10^9$/l)	Lym.# (µl)	Neut.# (µl)
I	Control (distilled water)	16.07±0.15	7.76±0.20	7.8±0.10
II	169.7 (low dose)	14.10±1.65 *	11.10±1.70*	2.53±0.24*
III	339.4 (Middle dose)	11.97±2.52 *	9.87±2.34	1.70±0.25*
IV	509.1(High dose)	10.57±1.14 *	7.93±1.27*	2.23±0.62*

Results were expressed as mean values ± standard error of mean. *P=0.05 when compared with the control group. Sample size (n=5). WBC= White blood cell count, Lym.# = absolute count of lymphocytes and Neut.# = absolute count of neutrophils.

Table 5 : Effect of 21 days oral administration of fractions of *Bixa orellana* leaves on haematological parameters in albino rats.

Group	Treatment (mg/kg)	WBC ($x10^9$/l)	Lym.# (µl)	Neut.# (µl)
I	339.4 (aqueous)	15.23±0.3*	8.01±0.15*	3.20±0.09*
II	339.4 (butanol)	6.80±0.05 *	4.73±0.13*	1.80±0.10*
III	339.4 (ethyl-acetate)	9.54±1.20 *	7.90±0.32*	4.10±0.30*
IV	339.4 (chloroform)	9.67±0.17 *	7.93±1.27 *	2.23±0.62*
V	339.4 (n-hexane)	9.70±0.15*	8.07±0.14*	2.37±0.12*

Table 6 : Effect of 21 days oral administration of ethanol extract of *Bixa orellana* leaves on haematological parameters in albino rats.

Group	Treatment (mg/kg)	PLT ($x10^9$/l)	MPV (fL)	PDW (%)	P-LCR (%)
I	Control (distilled water)	842.67± 55.05	7.60±0.08	9.04±0.03	9.90±0.17
II	169.7 (low dose)	718.33± 61.62*	7.70±0.15	9.16±0.01	10.16±0.10
III	339.4 (Middle dose)	674.33±57.65*	7.80±0.1	9.30±0.04	10.80±0.18*
IV	509.1 (High dose)	703.67±28.60*	7.70±0.50	9.60±0.03	12.03±0.14*

Results were expressed as mean values ± standard error of mean. *P=0.05 when compared with the control group. Sample size (n=5). PLT= platelet count, PDW= platelet distribution width, MPV= mean platelet volume and P-LCR= large platelet ratio.

Table 7 : Effect of 21 days oral administration of fractions of *Bixa orellana* leaves on haematological parameters in albino rats.

Group	Treatment (mg/kg)	PLT ($x10^9$/l)	MPV (fL)	PDW (%)	P-LCR (%)
I	339.4 (aqueous)	812.67± 3.50*	7.70±0.30	9.23±0.07	10.40±0.17
II	339.4 (butanol)	682.33± 6.39*	7.60±0.15	10.5±0.01	12.33±0.10*
III	339.4 (ethyl-acetate)	662.33±14.15*	7.80±0.60	9.20±0.04	12.33±0.18*
IV	339.4 (chloroform)	754.67±8.20*	7.70±0.50	9.30±0.03	12.30±0.14*
V	339.4 (n-hexane)	819.33±16.67*	7.80±0.15	9.4±0.05	11.86±0.10*

Table 8 : Effects of 21 days oral administration of ethanol extract of Bixa orellana leaves on serum electrolyte concentration in albino rats.

Group	Treatment (mg/kg)	Na (mmol/)	K (fL)	HCO_3 (%)	Cl^- (%)
I	Control (distilled water)	141.60± 0.50	4.30±0.10	24.00±0.02	101.0±0.08
II	169.7 (low dose)	141.30±1. 74	4.75±0.27 *	24.01±0.10	100.0±0.01
III	339.4 (Middle dose)	139.17± 0.97*	4.73±0.03 *	22.00±0.56	103.05±0.50
IV	509.1 (High dose)	138.67±0.45*	4.17±0.09	23.00±0.06	99.0 ±0.10

Results were expressed as mean values ± standard error of mean. *P<0.05 when compared with the control group. Sample size (n=5). Na= Sodium, K= potassium, HCO_3= bicarbonate and Cl^-= chloride

Table 9 : Effects of 21 days oral administration of fractions of Bixa orellana leaves on serum electrolyte concentration in albino rats.

Group	Treatment (mg/kg)	Na (mmol/)	K (fL)	HCO_3 (%)	Cl^- (%)
I	339.4 (aqueous)	140.67± 0.62	4.39±0.08	22.0±0.05	102.05±0.50
II	339.4 (butanol)	138.0± 0.57*	4.20±0.50	23.0±0.03	101.1±1.0
III	339.4 (ethyl-acetate)	139.77±0.46*	4.31±0.02	20.0±0.01	100.0±0.80
IV	339.4 (chloroform)	140.30±0.50	4.51±0.04*	24.0±0.18	98.0±1.40
V	339.4 (n-hexane)	142.33±0.80	4.67±0.04*	22.0±0.05	104.0±1.80

Phytochemical screening showed that the leaves of *Bixa orellana* contains flavonoids and tannins in abundance. Phenolics have been used since early twenties for the destruction of cancer cells and for their neuro-protective, antihypertensive, antitumor, etc. effects (Zheng and Wang, 2001). Results obtained by Kee *et al.* (2008) indicated that tannins have major part to play in anticoagulant activity of some plant extracts. Several of the plants extracts they used for their research were found to exhibit anticoagulant/antithrombotic activity, but after removal of tannin there was no significant activity recorded for most of the tested extracts. Flavonoids are known to have anti-oxidant properties. The erythropoietic effect of *Bixa orellana* leaves may be related to its anti-oxidant property of the flavonoids. The anti-oxidant activity of the flavonoids may protect both the haematopoietic committed stem cells and the formed blood cells from the attack of reactive free radicals in the body (Uboh *et al*., 2010). Cardiac glycosides and Saponins were present in moderate amount in leaves of *Bixa orellana*. Cardiac glycosides are useful in management of cardiovascular disease (Trease and Evans, 2009).

Saponins are present in moderate amount in *Bixa orellana* leaves. Saponins are amphipathic glycosides grouped by the soap-like foaming they produce when shaken in aqueous solutions, and by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative.

The significant (P=0.05) increase in RBC, Hb conc. and PCV could be due to the diuretic effect attributed to the leaves of *Bixa orellana*, the methanol extract of the leaves of *Bixa orellana* has been reported to show significant diuretic activity by increasing the total volume of urine and levels of sodium, potassium and chlorine in urine (Radhika *et al*., 2009). Diuretics have been reported to cause decrease in oxygen known as hypoxia (Lullman *et al*., 2000). Hypoxia stimulates secretion of erythropoietin which in turn stimulates bone marrow production of RBC. The diminished blood volume caused by diuretics brings about a decrease in renal oxygen concentration which in turn jeopardises renal blood flow. Inability of the blood to deliver enough oxygen to the highly oxygen-consuming tubular cells of the kidney stimulates erythropoietin formation. Cardiac glycosides are also known to increase oxygen demand of tissues, hence causing hypoxia which induces erythropoiesis (Evans, 2009).

The anti-platelet effect of *Bixa orellana,* as shown by significant (P=0.05) decrease in PLT, supports the use of the drug as anti-venom for treatment of snake-bites in traditional medicine. Some of the factors associated with traditional medicine practices for snakebites in Africa are social problems, geographical difficulties, and the insufficient production, supply or distribution of anti-venoms. Therefore, experimental validation of the traditional use of plants in treatment of snakebite is important and can facilitate the development of low-cost phytotherapeutic agents (Elisabetsky, 1991). The ethanol extract of the leaves of *Bixa orellana* has inhibited 100% the effects of poisoning produced by snakebite and inhibitory activity against the enzyme prostaglandin synthetase at a concentration of 750ug/ml (Terashima *et al*., 1991); ethanol extract of the leaves has shown highly significant activity against the edema-forming, defibrinating and coagulant effects of *Bothrops asper* venom in Swiss Webster mice (Nunez *et al.*, 2004). Snake venom contains proteolytic enzymes which enhances blood clotting by activating the clotting factors (Lullman *et al*., 2000). Venoms of vipers such as *Bothrox asper* have *in-vitro* coagulant effect and *in vivo* defibrinating effect on human plasma. As a haemostatic agent (procoagulant) snake venom causes toxic thrombosis i.e. coagulation of blood inside the blood vessels or intravascular blood clotting. The anti-platelet activity of *Bixa orellana* inhibits this coagulation; this justifies its usage in treatment of snake bite, coupled with its anti-inflammatory effect as indicated by the significant (P=0.05) decrease in WBC.

The significant (P=0.05) decrease in PLT observed from this study could be explored for its benefits. Antiplatelet agents are considered a significant tool in the treatment and/or prevention of cardiovascular thrombotic diseases (DeMeyer *et al*., 2008). Mechanistic studies indicate that antiplatelet agents significantly reduce the prevalence of primary and secondary coronary events related to cardiovascular disease (CVD). According to findings published in *Fitoterapia,* polyphenol –rich extracts from berries of *Aronia melanocarpa* and *Vitis vinifera* seem to be promising dietary supplements to prevent thrombosis in pathological states where plasma coagulant activity is observed, example, in hyper-cysteinemia or in

postmenopausal women after hormone therapy. The platelet anti-aggregant activity of 17 aqueous extracts of plants traditionally used in Guatemala for the treatment of 'blood disorders' and parasitic infections were assayed by Villar *et al.* (1997). The aggregation of washed human platelets induced by thrombin (0.075 U/mL) was inhibited by extracts of seven of the plants screened and *Bixa orellana* was among the seven. In this study, ethanol extract and fractions of *Bixa orellana* leaves showed significant (P=0.05) decrease in total WBC count, an effect known as leukopenia. The underlying mechanism could be immune-mediated or hypersensitivity reaction. Although there is a significant decrease in the total WBC count and absolute neutrophil count, there is a correspondent significant increase in the absolute lymphocyte count. Lymphocytes promote growth of erythropoietic cells by secretion of interleukins, a haematopoietic growth factor (Cox, 2009). Results obtained from the study of the effect of the ethanol leaf extract and fractions of *Bixa orellana* on serum electrolyte concentration revealed that, compared to the control group, there is a significant (P=0.05) increase in potassium concentration at low and middle doses of the ethanol extract and with the chloroform and n-hexane fraction and a significant (P=0.05) reduction in the concentration of sodium at middle and high doses of the ethanol extract, and with butanol and ethylacetate fractions. The decrease in sodium concentration may be due to the diuretic properties of *Bixa orellana* leaves as reported by Rhadika *et al.* (2009), since diuretics affect the serum electrolyte levels most (Lullmann, 2003). The distribution of electrolytes in body fluids is responsible for the electrical activity of the tissues including the myocardium (Guyton and Hall, 2005). Thus any change in the concentration of any of the electrolytes definitely alters the electrical activity of the cardiac muscle. Serum potassium and sodium ions play important role in cardiovascular activity.

IV. CONCLUSION

Bixa orellana leaf extract contains bioactive components that have significant effects on the various indices of the blood. The anti-platelet effect of the leaf might be of great pharmacological importance on further research. This property could also be beneficial in the treatment of cancer, since fibrin clot formation plays a vital role in masking tumours and promoting their attachment to the vascular endothelium, thereby aiding its progression.

V. REFERENCES

[1]. Adebayo, J.O., Adesokan, A.A., Olatunji, L.A., Buoro, D.O. and Soladoye, A.O. (2005). Effect of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. *Biokemistri*, 17 (1): 45-50.

[2]. Alexander, R. R. and Grifiths. J. M. (1993a). Haemotocrit In: *Basic Biochemical Methods*, 2nd Ed., New York: John Willey and Sons, Inc. Publications. pp. 186 –187.

[3]. Alexander, R. R. and Grifiths. J. M. (1993b). Haemotocrit determination by the cyanomethaemoglobin method In: *Biochemical Methods*, 2nd Ed, New York: John Willey and Sons, Inc. Publications. pp. 186 – 187.

[4]. AOAC, (2003). Official methods of analysis of the association of official's analytical chemists, 17th Edn. Association of official analytical chemists, Arlington, Virginia. pp. 96-105.

[5]. Chevellier, A. (1996). *The Encyclopedia of Medicinal Plants*. London: Dorling Kindersley Ltd., pp. 81-205.

[6]. Chopra, R. N. (1949). *Poisonous Plants of India*. Delhi, India. Pp. 203-210.

[7]. Cox, M. (2009). *Interpreting Blood Tests and Investigations*. RCN Conference, January, 2009.

[8]. DeMeyer, S. F., Vanhooelbeke, K. V., Broos, K., Salles, I. I. and Deckmyn, H. (2008). Antiplatelet drugs. *Brazilian Journal of Haematology*, 142: 515-528

[9]. De-oliveira, A.C., Silva, I.B., Manhaes-Rocha, D.A. and Paumgarrtten, F.J. (2003). Induction of liver mono-oxygenases by Annato and Bixin in female rats. *Brazilian Journal of Medical Biology Research*, 36: 113-118.

[10]. Elisabetsky, E. (1991). Sociopolitical, economical and ethical issues in medicinal plant research. *Journal of Ethnopharmacology*, 32: 235-239.

[11]. Elujoba, A. A., Odeleye, O. M. and Ogunyemi, C. M. (2005). Traditional Medicine Development for Medicinal and Dental Primary Health Delivery System in Africa. *African Journal of Traditional, Complementary and Alternative Medicine*, 2 (1): 466-468.

[12]. Etukudo, I. (2003). *Ethnobotany: Conventional and Traditional Uses of Plants*. Verdicts Press, Uyo. p.191.

[13]. Evans, W. C. (2009). *Trease and Evans Pharmacognosy*, 16thEdition. New York: Sauders Elsevier Limited. pp. 104-262.

[14]. Guyton, A. C. and Hall, J. E. (2005). *Textbook of Medical Physiology* (11th ed.,) Philadelphia: Elsevier Publishers pp. 417-450.

[15]. Joshi, P.K., Bose, M. and Harish, D. (2002). Changes in certain haematological parameters in siluroid catfish, *Clarias batrachus*Linn., exposed to cadmium chloride. *Pollution Resources*, 21 (2): 129-131.

[16]. Kee, N. L. A., Mnonopi, N., Davids, H., Naudé, R. J. and Frost, C. L. (2008). Antithrombotic /anticoagulant and anticancer activities of selected medicinal plants from South Africa. *African Journal of Biotechnology*, 7(3): 217-223.

[17]. Kulpmann, W. R. (1992). Determination of electrolytes in serum and plasma. *Wien Klin Wochenschr Suppl.* 192: 37-41.

[18]. Lewis, S. M., Bain, B. J. and Bates, I. (2001). Dacie and Lewis: practical haematology, Ninth Edition, London, New York: Elsevier, pp. 9-40.

[19]. Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. Arch Toxicol., 53: 275-289.

[20]. Lüllmann, H., Morh, K., Ziegler, A. and Bieger, D. (2000). *Color Atlas of Pharmacology*, 2nd ed., New York: Thieme Stuttgart. pp. 1-386.

[21]. Núñez, V., Otero, R., Barona, J., Saldarriaga, M., Osorio, R. G., Fonnegra, R., Jiménez, S. L., Díaz, A. and Quintana, J. C. (2004). Neutralization of the edema-forming, defibrinating and coagulant effects of *Bothrops asper* venom by extracts of plants used by healers in Colombia. Brazilian Journal of Medical and Biological Research, 37 (7): 969-977.

[22]. Pawar, R. S., Jain, A. P., Kashaw, S. K. and Singhai, A. K. (2006). Haematopoietic activity of *Asteracantha longifolia* on Cyclophosphamide-Induced Bone Marrow Suppression. *Indian Journal of Pharmaceutical Science*, 68: 337-340.

[23]. Rhadhika, B., Begum, N., Srisailam, K. and Reddy, V.M. (2009).Diuretic Activity of *Bixa orellana* L. Leaf Extracts. *Indian Journal of Natural Products and Resources*, 1 (3): 353-355.

[24]. Schultes, R.E. and Raffauf, R. (1990). *The Healing Forest: Medicinal and Toxic plants ofthe Northwest Amazonia*. Portland, Oregon. Pp 109-111.

[25]. Sofowora, E. A. (1993). *Medicinal plants and traditional medicine in Africa*. (2nd Ed.). Ibadan, Nigeria: Spectrum Books Ltd. pp. 181-204.

[26]. Terashima, S., Shimizu, M., Horie, S. and Morita, N. (1991). Studies on aldose reductase inhibitors from natural products. IV. Constituents and aldose reductsase inhibitory effect of *Chrysanthemum morifolium, Bixa orellana, and Ipomea batatas*. *Chemical Pharmocology Bulletin* 39: 3346-3347.

[27]. Uboh, F. E., Okon, I. E. and Ekong, M. B. (2010). Effect of Aqueous Extract of *Psidium guajava* Leaves on Liver Enzymes, Histological Integrity and Haematological Indices in Rats. *Gastroenterology Research*, 3 (1): 32-38.

[28]. Varley, H., Cowenlock, A. H. and Bell, M. (1980). *Practical Clinical Biochemistry*. 5th edition, London: Heinemann, Pp 771-799.

[29]. Villar, R., Calleja, J. M., Morales, C. and Caceres, A. (1997). Screening of 17 Guatemalan Medicinal Plants for Platelet Antiaggregant Activity. *Phytotherapy Research*, 2: 441-445.

[30]. World Health Organisation. (1996). *Guidelines for the Assessment of Herbal Medicines*. WHO Expert Committee on Specification for Pharmaceutical Preparation Technical Reports Series. No. 863, Geneva.

[31]. Zheng, W. and Wang S. (2001). Antioxidant activity of Phenolic compounds in selected Herbs. *J. Agric. Food Chem.* **49**: (11): 5165 – 5170.