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Table of Contents: Volume 10 Number 22 10 June, 2016

ARTICLES

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Full Length Research Paper

Toxicity and effects of fig (*Ficus carica*) leaf aqueous extract on haematology and some biochemical indices of wistar albino rats *(Rattus norvegicus)*

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The toxicity and effects of *Ficus carica* leaf aqueous extract on the haematology and some biochemical parameters were investigated in wistar albino rats for 4 weeks. Various doses (100, 200 and 400 mg/kg body weight) of the extract were administered orally to the rats. The haematological parameters were determined using a Neubauer-type hemocytometer with Toisson's solution as the diluting fluid for red blood cell (RBC) and Turk's solution for white blood cell (WBC). The biochemical parameters and glucose level were estimated using O-toluidine method. The acute toxicity LD_{50} could not be established from the range of 50 to 6000 mg/kg doses of the extract. The phytochemical analysis showed the presence of flavonoid, tannins, cardiac glycosides, steroids, and saponins, in plant with flavonoid and tannins in high and moderate abundance. Oral LD_{50} was therefore not determined because mortality was not observed. The packed cell volume (PCV), RBC and hemoglobin (Hb) were significantly increased with prolonged administration of the extract. There was dose and time dependent significant decrease ($p < 0.05$) in neutrophils when compared with the control in the experiment. The results indicated dose-related decreases in serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the test groups (9.88 ± 3.14^{b} to 24.67 ± 6.43^{a} and 19.00 ± 3.00^{a} to 99.33 ± 25.32^{a} U/L), respectively when compared with those of the ALT and AST control 15.03 ± 0.04^{a} to 39.67 ± 0.58^{b} and 30.33 ± 0.58^{d} to 126.0 ± 5.29^{a}, respectively. *F. carica* aqueous extract has hepatoprotective capability and could be used at an average and prescribed dosage in consumption and drug production for enhancement of erythrocytes and haemoglobin concentration.

Key words: *Ficus carica,* biochemical indices, haematology, albino rats, phytochemical screening.

INTRODUCTION

Plants have been the major source of drugs for medicine in most developing and established countries in the world (Obadoni and Ochuko, 2001). The use of plant extracts and phyto-chemicals, both with its known toxicity effects

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on haematological and biochemical parameters have been identified by the World Health Organization as alternative sources of therapeutic treatments (Ghasemi, 2014). Medicinal plants therefore remain significant as therapeutic remedies in many developing countries (Sibel et al., 2005). The beneficial medicinal effects of plant materials typically result from the combinations of their secondary metabolites; their medicinal actions are unique to particular plant species or groups of distinct taxonomy (Wink, 1999).

For many centuries, humans relied on medicinal plants to cure various diseases (Ramaa et al., 2006). Most plant parts such as the leaves and roots have been used for curative, antipyretic, purgative and treatment of other diseases. The acceptance of herbal remedy is gradually increasing worldwide (Inoue et al., 2011).

There is also growing concern about the toxicity and adverse effects of several hundreds of dietary herbs/vegetables which have remained either uninvestigated or poorly investigated and are increasingly used by patients with different diseases (Ghasemi, 2014). Therefore, in contradiction to their usefulness, some herbal remedies used in treatment of some diseases (liver diseases inclusive) may as well induce some toxic effects on the patient (Ghasemi, 2014). This specific toxic component of the plant most times is not known because of difficulty in proper analysis of the plant material especially if unsuspected (Ekpenyong et al., 2012).

According to several reports, other factors such as active and toxic components overdose/abuse, prolonged use, and allergies can also be attributed to the major causes of hepatotoxicity and other forms of toxicity. Apart from the safety and the acclaimed therapeutic efficacy, other reasons for the recent surge in consumption of herbal remedies included easy accessibility, more acceptability from cultural and spiritual perspectives and inadequacy of primary health care services. The importance of herbs and plant leaves remain highly significant in medicine. Thus, proper research on leaves and herbs used in herbal and normal medicine is important to check the adversity and usefulness of the leaves and herbs (Ekpenyong et al., 2012).

Plant-derived drugs thus served as a prototype to develop more effective and less toxic medicines. *Ficus carica* is one of the medicinally important plants that belong to the mulberry tree (Moraceae). The Moraceae leaves are well known because of its high antioxidant character but the actual active constituents responsible for this have not been determined despite several experiments. It is used in the traditional system of medicine for healing various diseases (Guarrera, 2003). It has been reported that the leaves are used in traditional medicine as laxatives, stimulant, against throat diseases, emollient and haemorrhoid treatment (Sibel et al., 2005).

The importance of blood in maintaining good health cannot be overstated. The Chinese described blood as the ‘mother of energy’ in the sense that it provides the basic materials and fluid substances that are required to nourish the life essence of our being; thus, blood is represented as a receptacle for sustaining life energy (Sheng, 2003). In addition, because the lifespan of blood cells is relatively short, the blood needs to be constantly replenished (Sheng, 2003; Nebedum et al., 2010).

In South Eastern part of Nigeria, the leaves of *F. carica* among others are considered as excellent natural herbal blood boosters used especially for debilitating conditions, acute blood loss and blood deficiency diseases (Ahmad et al., 2013). Aqueous extract of *F. carica* leaves were further found to induce a significant hypoglycemic effects in rats but the mechanism involved in this effect was not elucidated (Sibel et al., 2005).

Most research works carried out on *F. carica* have focused on its hypoglycemic, antioxidant and other therapeutic effects. Research works on the *F. carica* toxicity effects on haematology and liver enzymes (biochemical parameters) are scanty thus signifying the importance of this study. The objective of this study was to determine the toxicity of *F. carica* leaf aqueous extract and its effects on the haematology and some biochemical parameters (liver enzymes) on albino rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Plant

F. carica leaves were collected from International Centre for Ethno-medicine and Drug Preparation in Nsukka, Enugu State, Nigeria. The leaves were identified and authenticated by Dr Nzekwe at the Department of Plant Science and Biotechnology, University of Nigeria. A voucher number UNN-EGACC, protocol no. 0330/2013 specimen was deposited in the herbarium.

Preparation of crude extract

F. carica leaves were thoroughly washed, shade-dried for 7 days and ground in an electric blender, Moulinex Master Chef Delicio (Type DFB1). Each crude extract was filtered through Whatman No. 3 filter paper and the filtrate concentrated *in vacuo* using a rotor evaporator for solvent evaporation. Dried extract was reconstituted in 10 ml dimethyl sulphoxide (DMSO) prior to use. The *F. carica* leaves extracts were prepared according to the method described by Nebedum et al. (2010). The homogenate was suspended in absolute ethanol for extraction, and then centrifuged, filtered through Whatman No. 1 filter paper and further re-centrifuged through Whatman No. 3. This was then rotor-evaporated to remove the ethanol before suspending in DMSO. The final concentration of *F. carica* extract in DMSO was determined by subtracting the weight of insoluble material from the weight of original sample to give 64% (w/v) of extract. The filtrate was then kept at -70°C until used.

Phytochemical screening

Qualitative phytochemical analysis of plant extracts was carried out according to the method described by Trease and Evans (1978) as the following.

Flavonoids

Lead acetate test: briefly, 2 .0 ml of each ethanolic extract was added 10% lead acetate solution and observed for a coloured precipitate indicative of flavonoids.

Tannin

To 2.0 ml of distilled water was added 0.1 g of each extract, boiled for 2 min, filtered and allowed to cool. To this was then dispensed 5% ferric chloride ($FeCl_3$) solution drop-wise and observed for colour change.

Saponins

The presence of saponins was assayed by the frothing test. Approximately, 5 mg of extract was added into 5 ml of distilled water, shaken vigorously and observed for persistent frothing.

Alkaloid

Approximately 2.0 ml of each ethanolic extract was added to 5 ml of 2% hydrochloric acid and allowed to steam in a water bath for 20 min, and then filtered. To 1 ml of each filtrate was dispensed 2 drops of Dragendroff's reagent (bismuth potassium iodide solution) and observed for precipitation.

Steroids

The Salkowski's test was used in testing for steroids. Approximately, 5 ml of each ethanol extract was evaporated to dryness and the residue re-dissolved in 5.0 ml of anhydrous chloroform and then filtered. To each filtrate in a 10 ml test tube was carefully dispensed 2.0 ml of concentrated sulphuric acid until a bi-layer was formed. The interface was then observed for a reddish brown coloration indicative of steroid. About 2 ml of each extract was further dried by evaporation, and to this was dispensed 2 ml of anhydrous chloroform and filtered. To a 2 ml filtrate was simultaneously added 2 ml acetic anhydride and 2 ml concentrated H_2SO_4 and carefully observed for the appearance of a blue-green ring indicating the presence of terpenoids.

Cardiac glycosides

The Keller-Kiliani test was adopted; to a 2 ml filtrate was dispensed 1 ml glacial acetic acid, $FeCl_3$ and concentrated H_2SO_4, and observed for green-blue coloration indicative of cardiac glycosides.

Experimental animals

A total of 180 wistar healthy albino rats of both sexes weighing 100 and 185 g used in this research work were obtained from the animal house of Department of Zoology, University of Nigeria, Nsukka. The animals were acclimatized for 2 weeks in their respective cages. The cages were kept in a well-ventilated and conducive room in the animal house inside the zoological garden. The animal were housed within the facility and maintained on grower's vital feeds and water *ad libitum*. Care and handling of the albino rats were done in accordance to the protocols described by the Animal Care Committee (UNN-EGACC) of University of Nigeria, Nsukka.

Toxicity and lethality (LD_{50}) test

The oral toxicity study was carried out using fixed dose method. The LD_{50} of aqueous extracts of the plant was determined using a total of 132 wistar rats of both sexes. The rats were fasted for 12 h and divided into the first groups of 2, 3, 4 and 5 which received an administration of 50, 100, 500 and 1000 mg/kg body weight of the extract, while in an another first phase of 2, 3, 4 and 5 groups were administered with 2000, 3000, 4000 and 5000 mg/kg body weight, respectively for 24 h. In another second phase that was observed for 48 h, the dosages were increased to 5500, 5750 and 6000 mg/kg. The rats were closely observed for toxic symptoms and behavioural changes for the first 2 h after extract administration. The LD_{50} was calculated as the geometric mean of the dose that resulted in 50% mortality (Shrivastava, 2012).

Experimental design

The wistar rats were divided into four groups and each group had 3 replicates of 4 rats: Group 0: control received distilled water orally only; Group 1: received 100 mg/kg of body weight of the extract; Group 2: were administered with 200 mg/kg body weight; and finally Group 3: were fed with 400 mg/kg body weight.

The animals were housed within the facility and maintained on grower's vital feeds and water *ad libitum*. The administration of this extract lasted for four weeks while haematological and biochemical studies were carried out weekly for 4 weeks of the experiment as well.

Estimation of haematological parameters

The haematological parameters were determined using a Neubauer-type hemocytometer with Toisson's solution as the diluting fluid for red blood cell (RBC) and Turk's solution for white blood cell (WBC) (Saravanan et al., 2012). The haemoglobin level of blood was estimated following the cyanmethaemoglobin method with some modifications. Each blood sample (0.02 ml) was mixed with 4 ml Drabkin's solution and allowed to stand for 10 min for proper colour development after which absorbance was read at 540 nm in Unican spectrophotometer against a blank. Haematocrit was analyzed by centrifugation of the blood using a microhaematocrit centrifuge (Hawkesley and sons, Ltd., Lancing, UK) at room temperature. The haematocrit was read after centrifugation using the micro haematocrit reader and the result expressed as the percentage of the whole blood. Haematological parameters such as MCHC, MCH and MCV were calculated according to Dacie and Lewis (2001):

MCHC (g/dl) = Hb (g/dl) PCV (%) × 100

MCH (pg/cell) = Hb (g/dl) × 10 RBC count in millions/mm^3

MCV (fl/cell) = PCV (%) × 10 RBC count in millions/mm^3

where MCHC is the mean corpuslar haemoglobin concentration, MCH is the mean corpulsar haemoglobin, PCV is the packed cell volume, Hb is the haemoglobin concentration, RBC is the red blood cell, pg is the pictograms, and fl is the femtolitres.

Estimation of biochemical parameters and glucose level

These were estimated using O-toluidine method (Sadauskas et al., 2011). Blood samples were collected from the eyes using haematocrit tubes by carefully inserting the tube into the median cantus to puncture the vein thus enabling the flow of blood into

Table 1. Phytochemical analysis of *F. carica* aqueous extract.

Phyto-constituents	Relative abundance
Alkaloids	+
Flavonoids	+++
Tannins	++
Saponins	++
Steroids	++
Cardiac Glycosides	++

+ = low in abundance; ++ = moderate in abundance; +++ = high in abundance

plain tubes (no EDTA anticoagulant) and these were allowed to clot and the serum separated by centrifugation. The serum collected is then subjected to biochemical assays. This was carried out according to the protocols described by Sadauskas et al. (2011) which is internationally accepted.

The following liver biochemical parameters analyzed were aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) from the serum obtained from the blood sample. The ALT was determined by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenyl hydrazine, while AST was determined using concentrations of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl hydrazine. ALP was determined by the method described by Englehardt (Sadauskas et al., 2011).

Glucose level was estimated using Otoluidine method (Nwani et al., 2014), while total protein was estimated spectrophotometrically using bovine serum albumin as a standard following the methods (Sadauskas et al., 2011).

Statistical analysis

All values were expressed as mean ± standard deviation (SD). The differences were compared using analysis of variance (ANOVA) followed by Dennett's test. P values< 0.05 were considered as significant.

RESULTS

Phytochemical screening

The flavonoid contents were high in abundance (+++), while other constituents such as the cardiac glycosides, steroids, saponins, and tannins were lower in abundance (++). Similarly, the alkaloids were the lowest (+) as elucidated in Table 1.

Toxicity studies

The acute toxicity LD_{50} could not be established from the range of 50 to 6000 mg/kg body weight doses of the extract. There was no mortality recorded when all the doses of the extract were administered orally to the rats. The rats showed negative behavioural changes at 5000,5500,57250 and 6000 mg/kg dosages. Oral LD_{50} was therefore not determined, because mortality was not observed. The LD_{50} obtained was higher than 6000 mg/kg by implication.

Haematological and morphological parameters

Results of the haematological parameters of the control and experimental groups are presented in Table 2. The RBCs count and haemoglobin in the experimental groups was significantly different from the control ($p< 0.05$) throughout the duration of the experiment and was significantly increased ($p<0.05$). There was significant difference in PCV values between the control and treated rat on day 7 which subsequently increased significantly ($p<0.05$) from day 14 of exposure. *F. carica* induced dose and time dependent significant increase in WBC count from day 14 onward ($p<0.05$) were observed, while values of blood parameters (MCV, MCH and MCHC) in the experimental rat were not significantly different ($p < 0.05$) from the control group throughout the duration of the experiment. Changes in the mean values of the leukocyte differentials are presented in Table 3. There was dose and time dependent significant decrease ($p < 0.05$) in the levels of neutrophils when compared with the control throughout the experimental duration. The lymphocyte levels were significantly elevated ($p< 0.05$) from day 7 onward, but the values of the monocytes, basophils and eosinophils were not significantly different ($p<0.05$) from the control.

Biochemical parameters

The activities of the enzymes assayed as well as the concentrations of other biochemical parameters are shown in Table 4. The results indicated dose-related decreases in serum activities of ALT and AST in the test groups (9.88 ± 3.14^{b} to 24.67 ± 6.43^{a} and 19.00 ± 3.00^{a} to 99.33 ± 25.32^{a} U/L), respectively when compared with those of the ALT and AST control 15.03 ± 0.04^{a} to 39.67 ± 0.58^{b} and 30.33 ± 0.58^{d} to 126.0 ± 5.29^{a}, respectively.

Table 2. Effects of exposure to various concentration levels of aqueous extract of *F. carica*on haematological profile of albino rats

Parameter	Conc. (mg/kg)	Duration (Days)			
		7	14	21	28
PCV (%)	Control	41.67±0.58[a]	40.33±0.58[a]	32.33±2.52[a]	42.33±1.53[a]
	100	39.67±2.52[a]	42.33±2.52[a, b]	40.33±1.53[b]	42.67±2.52[a]
	200	39.67±5.86[a]	43.33±5.86[b]	42.00±6.25[b]	43.00±1.00[a, b]
	400	39.33±4.04[a]	46.33±1.53[a]	43.33±2.89 [b]	46.00±1.00 [b]
Hb (g/dl)	Control	13.67±5.08[a]	9.1±0.10[a]	5.25±0.18[a]	10.90±0.26[as]
	100	13.77±1.02[a]	12.5±1.10[b]	13.33±0.64[b]	12.50±1.37[a]
	200	13.83±0.96[a]	12.47±1.68[b]	13.13±0.78[b]	12.33±1.47[a]
	400	13.33±1.27[a]	13.43±1.10[b]	14.10±0.52[b]	13.03±1.10
WBC (mm3) x103)	Control	9905.0±5.0[a]	10066.67±57.74[a]	9366.7±57.73[a]	9533.3±57.73[a]
	100	10900.0±4.35[b]	9800.0±360.56[a]	0466.7±814.5[a]	10333.3±404.2[a,]
	200	10566.7±351.2[a,]	9700.0±173.2[a]	10666.7±404.15[a]	0066.7±152.6[a,b]
	400	10433.3±757.19[a,]	10066.67±611.0[a]	10566.7±1193.035[a]	10500.0±854.4[b]
RBC (mm3) x 106	Control	11.65±0.57[a]	9.44±0.037[a]	8.42±0.03[a]	10.49±0.006[a]
	100	9.9±1.81[a]	11.12±0.37[b]	10.25±0.76[b]	10.78±0.42[a,b]
	200	10.72±1.87[a]	11.65±0.29[b]	10.79±0.06[b,c]	12.33±1.47[a]
	400	10.46±1.08a	11.46±0.52b	11.43±0.48c	11.53±0.34[c]
MCV (M^3)	Control	35.82±2.09[a]	42.74±0.694[b]	38.40±2.886[a]	40.36±1.452[a]
	100	40.42±5.77[a]	38.14±3.005[a]	39.58±4.550[a]	39.56±1.022[a]
	200	37.14±1.31[a]	37.18±0.390[a]	38.90±5.658[a]	38.31±2.567[a]
	400	37.68±2.60[a]	40.45±1.527[a,b]	37.90±1.475[a]	39.94±1.917[a]
MCHC (%)	Control	32.79±0.935[a]	22.56±0.164[a]	16.20±1.412[a]	25.75±0.327[a]
	100	34.70±1.345[a]	29.64±3.894[b]	33.06±0.862[b]	29.49±4.911[a]
	200	37.14±1.31[a]	28.83±4.41[b]	31.56±3.162[b]	28.68±3.36[a]
	400	33.92±0.624[a]	29.02±2.633[b]	32.70±3.510[b]	28.32±1.903[a]
MCH (pg/cell)	Control	11.76 ± 0.942[a]	9.593±0.192[a]	6.197±0.254[a]	10.40±0.251[a]

Values in rows with different superscript letters (a, b, c, d and e) differ significantly ($p<0.05$)

However, the groups fed 200 mg kg^{-1} body weight and 400 mg kg^{-1} body weight extracts had significantly lower ALT activities of 22.67±9.87[a] and 20.67±14.47[ab] U/L, respectively relative to those of the control 39.67±0.58[b] and 15.03±0.04[a]. There were mild variations in the ALP activities, 73.60±31.87[a] to 305.6±24.66[a] U/L of the experimental rats when compared with the ALP control of 38.07±0.16[b] to 332.1±0.90[a] as were also variations in the ALT/AST ratios of the experimental animals (0.22±1.557[c] to 0.55±0.445[a]) when compared with the control of 0.12±0.07[c] to 0.56±0.051[a], respectively.

There were mild alterations in the serum concentrations of total proteins (85.08±0.09[a] to 92.00±5.0[a] gL^{-1}). There were very slight dose-dependent decreases ($p>0.05$) in the serum glucose concentrations of the test rats (3.01±0.07[a] to 3.58±0.154.68[a] $mmoL^{-1}$) when compared with those of control rats 4.69±0.90[a] to 4.90±0.07[a] $mmoL^{-1}$.

DISCUSSION

Phytochemical screening

Preliminary phytochemical investigations revealed the presence of alkaloids, saponins, tannins, and cardiac glycosides similar to the findings of such extract of *F. carica* (Janardhanan, 2000). Some of the biological functions of flavonoids include protection against allergies, free radicals, platelet aggregation of microorganisms, ulcer, hepatotoxins and tumours (Okwu, 2004; Nebedum et al*.,* 2010). Saponins on the hand have the properties of precipitation of proteins, cholesterol-binding, and haemolysis (Fatemi et al., 2007). Other phyto components such as alkaloids and glycosides found in this plant also did not have properties relating to increased haemapotesis. Furthermore, the behavioural components observed during exposures to the extract in

Table 3. Effects of exposure to various concentration levels of aqueous extract of *F. carica* on differential WBC counts (percentage) in albino rats.

Types of leucocytes	Conc. (mg/kg)	Duration (Days)			
		7	14	21	28
Neutrophils	Control	28.67±0.58[a]	28.67±0.58[a]	15.00±1.00[a]	24.33±3.2
	100	32.67±4.62[a]	26.0±8.72[a]	27.33±6.43[b]	26.33±5.50[a]
	200	31.67±4.04[a]	24.33±6.66[a]	23.67±5.51[a,b]	27.33±4.51[a]
	400	28.67±5.03[a]	28.33±2.08[a]	25.00±5.00[b]	25.33±6.43[a]
Lymphocytes	Control	67.67±0.58[a]	68.67±0.58[a]	81.00±1.00[a]	70.00±1.00[a]
	100	65.33±3.06[a]	72.0±7.21[a]	72.33±5.86[a]	72.00±5.29[a]
	200	67.33±3.06[a]	74.0±6.25[a]	7567±5.86[a]	71.33±3.06[a]
	400	68.67±5.03	71.33±2.31[a]	75.00±5.00[a]	74.33±6.81[a]
Monocytes	Control	1.00±0.00[a]	0.67±0.58[a]	0.00±0.00[a]	-
	100	1.33±1.15[a]	0.67±0.58[a]	0.00±0.00[a]	0.67±0.58[a]
	200	0.33±0.58[a]	1.0±1.0[a]	0.67±0.58[b]	1.00±1.00[a]
	400	1.33±1.15[a]	0.00±0.00[a]	0.00±0.00[a]	-
Basophils	Control	1.00±0.00[a]	-	-	-
	100	0.33±0.58[a]	0.67±1.15[a]	-	0.67±0.58 [a]
	200	0.33±0.58[a]	0.33±0.58[a]	-	0.33±0.58 [a]
	400	0.67±0.58[a]	-	-	-
Eosinophils	Control	1.00±0.00[a]	0.67±0.58[a]	0.67±0.58[a]	0.67±0.58[a]
	100	0.33±0.58[a]	0.67±0.58[a]	0.33±0.58[a]	0.33±0.58[a]
	200	0.33±0.58[a]	0.33±0.58[a]	0.00±0.00[a]	-
	400	0.67±0.58[a]	0.33±0.58[a]	0.33±0.58[a]	0.33±0.58[a]

Values in rows with different superscript letters (a, b, c, d and e) differ significantly ($p<0.05$)

this investigation were dose-dependent somnolence and sedation which could easily be likened to side effects of the active constituents (flavonoid and saponins) of the extract (Ekpenyong et al*.,* 2012). However, studies are needed to determine the effect of the extract on humans since it is popularly used in folk medicine for seizure control, level of consciousness and blood pressure.

Haematological parameters

Time-dependent increases in haemoglobin red blood cells and PCV imply that the extracts may enhance the populations of red blood cells produced from the bone marrow, as well as increase the oxygen-carrying capacity of the whole blood because of the increased number of red blood cells in the blood (Fatemi et al*.,* 2007).

From the results obtained during the experiment, the PCV and the Hb were observed to have increased significantly. This indicated that the *F. carica* aqueous extract is not toxic because decrease in PCV and Hb concentration shows toxicity to the red blood cells. The RBC count was observed to have increased significantly from the second week of extract administration to the last day of blood analysis. This showed that the *F. carica* extract increased the RBC and this agreed with Nebedum et al*.* (2010) who reported on *F. carica* as an excellent blood builder. It also supports the traditional use of the *F. carica* as a blood enhancer.

Biochemical parameters

The decrease in the serum AST activities and ALT/AST of the test groups when compared with the control are not significant ($p>0.05$). Serum ALT/AST has been used as an index to monitor liver pathology (Eteng et al., 1998; Akinloye and Olorede, 2000). Ratios higher than unity are indicative of adverse pathological effect on the liver. From the studies, it has been shown that infusion of *F. carica* leaves maintain the ALT/AST ratios at favourable levels. Serum ALP is a sensitive detector for intrahepatic and extrahepatic bile obstruction, the presence of infiltrative diseases of the liver and all bone diseases associated with osteoblastic activity, for example, osteomalcia and rickets among others (Mayne, 1994; Vasudevan and Sreekumari, 2005). From the results obtained, it is likely that the concentrations of *F. carica* leaf extract used in

Table 4. Effects of exposure to various concentration levels of aqueous extract of F. *carica* on some biochemical indices in albino rats

Biochemical parameters	Conc. (mg/kg)	Duration (days)			
		7	14	21	28
AST	Control	30.33±0.58[d]	96.67±4.16[a]	126.0±5.29[a]	109.67±0.58[b]
	100	22.00±1.73[a]	79.67±5.03[a]	99.33±25.32[a]	80.00±5.00[a]
	200	20.00±1.73[b]	64.33±5.13[a]	91.67±14.50[a]	76.67±7.64[a,b]
	400	19.00±3.00[a]	60.0±36.06[a]	89.67±17.79[a]	72.33±17.56[a,b]
ALT	Control	15.03±0.04[a]	39.67±0.58[b]	36.00±1.00[b]	33.00±1.00[b]
	100	12.08±2.11[b]	24.67±6.43[a]	23.33±6.43[a]	19.33±4.16[a]
	200	11.01±1.46[b]	22.67±9.87[a]	20.33±4.16[a]	16.00±6.00[a]
	400	9.88±3.14[b]	20.67±14.47[a,b]	17.00±12.49[a]	15.00±6.00[a]
ALT/AST Ratio	Control	0.12±0.074[c]	0.451±0.107[a]	0.56±0.051[a]	0.39±0.048[a]
	100	0.55±0.445[a]	0.31±0.590[a],	0.23±1.280[b]	0.24±1.994[b]
	200	0.39±0.168[a]	0.29±1.046[b]	0.25±1.186[b]	0.28±0.935[a,b]
	400	0.30±0.707[b]	0.41±0.221[a]	0.22±1.557[c]	0.30±0.568[a,b]
ALP	Control	82.20±1.04[a]	38.07±0.16[b]	332.1±0.90[a]	110.43±0.35[a]
	100	83.53±1.63[a]	92.87±15.23[a,b]	264.5±66.23[a]	110.4±27.60[a]
	200	106.80±20.04[a]	73.60±31.87[a]	305.6±24.66[a]	116.5±54.27[a]
	400	93.00±15.14[a]	100.6±41.38[a,b]	286.47±38.48[a]	165.6±99.51[a]
Glucose	control	4.71±0.50	4.90±0.07	4.70±041	4.69±0.90
	100	3.58±0.15	3.31±0.57	3.41±0.61	3.29±0.86
	200	3.20±0.81	3.16±0, 21	3.07±0.01	3.11±0.02
	400	3.17±0.06	3.02±0.02	3.01±0.07	3.08±0.03
Protein	control	89.00±0.21	88.61±0.03	87.57±0.61	86.10±0.07
	100	92.00±5.0	91.02±01	89.03±0.04	88.03±0.09
	200	88.01±0.60	89±0.07	88.04±0.01	87.08±0.01
	400	87.01±0.01	86.07±0.05	88.03±0.02	85.08±0.09

Values in rows with different superscript letters (a, b, c, d & e) differ significantly ($p<0.05$).

this study did not adversely interfere with the calcification and/or metabolic activities involving the liver.

There were decreases in the serum glucose concentrations of the test rats (in a dose-dependent manner) when compared with those of control rats. Aqueous infusions of some medicinal plants have been reported to cause hypoglycemia in rats by increasing the level of insulin in the blood (Edem and Usoh, 2009). Previous reports have indicated a significant reduction in blood glucose when crude metanolic extracts of *F. carica* leaves were administered to rats (Osadebe and Ukweze, 2004) and a significant reduction in blood had also been reported (Lamela et al., 1985, 1986; Eno and Itam, 1996; Kako et al., 1996; Akinloye and Olorede, 2000; Svetlov et al, 2006). The observed differences in glucose-lowering effects could be attributed to the type of solvent used in preparing the extracts in these reports.

It can be stated that *F. carica* aqueous extract does not have any adverse effect on the liver or blood constituents; rather it could be a blood enhancer and builder.

Conclusion

Researches in herbal medicine have attained an incredible level in recent past. The applications have received greater attention as an alternative to clinical therapy leading to increasing demand. The results obtained from analysis of some haematological and biochemical profile (some plant constituents) in pharmaceutical industries have gone a long way in the elevation of the status of the traditional herbal medicine in Africa and Nigeria in particular. Hence, herbal medicines'

MCH, MCV, HB, ALT and AS activities on *F. carica* treated rats have shown that this species has no adverse effect on the liver or blood constituents and possess no or low hepatotoxic activity.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Phytochemical screening of *Xylopia aethiopica* with emphasis on its medicinally active principles

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This work aimed at investigating the functional types and compositions of bioactive compounds in *Xylopia aethiopica*. Plant parts (petiole, leaf, seed, stem bark, and roots) were collected from Vandeikya Local Government Area of Benue State, North Central Nigeria. Extracts were qualitatively screened following standard protocols. Phytochemicals were screened and tested. This was followed by quantitative analysis of selected bioactive compounds using double extraction gravimetric method. Data were statistically analysed using SPSS software. From the result, alkaloids, saponins, tannins, reducing sugar, anthraquinones, steroids, flavonoids, and glycosides were present in the parts investigated. The stem bark contained very high amount of saponin (8.33%), alkaloid (5.67%) and flavonoid (5.24%). The seed had moderate amount of the quantified compounds. The high positive correlation between flavonoid and alkaloid (+0.999) was the most significant value (p=0.022) obtained though the quantified chemicals were all positively correlated. Therefore, based on quantity, the bark is the most important part of medicinal importance followed by the seed. Based on functional types of phytochemicals, the petiole is the most important part which contains 7 out of 9 active principles screened, followed by the bark and the leaf. Therefore, the tree could serve as a source of making different types of cheap multifunctional drugs.

Key words: Extracts, bioactive compounds, phytochemicals, correlation, drug, *Xylopia aethiopica.*

INTRODUCTION

Xylopia aethiopica is a tree of more than 20 m of height and 60 to 75 cm in diameter. It grows in the forest zone and especially along the rivers and in arid areas. The fruit is a slightly hooked cylindrical pod reaching 2 to 3 mm in width. The mature fruits of green colour take a brown - black colouration after drying and they are commonly used as spices. Numerous research studies have confirmed the spice's anti-inflammatory and antipyretic (fever reducing) properties (Karawya et al., 1979; Fleischer, 2003).

In Nigeria, fruits extracts and stem bark decoction are used in the treatment of bronchitis, biliousness and dysentery (Asekun and Adeniyi, 2004). Fruits are used to counter pain and as a carmative and laxative and they are suspected to enhance fertility and aid delivery (Karawya et al., 1979; Asekun and Adeniyi, 2004). *X. aethiopica* dried fruit and bark are used to treat asthma, arthritis, neuralgia and chronic pain (Karawya et al.,

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Table 1. Qualitative screening of active principles in different plant parts.

Plant part	Alkaloids	Saponins	Tannins	Reducing sugar	Phloba-tannin	Anthraquinone	Steroids	Flavonoids	Glycosides
Petiole	+	+	+	-	-	+	+	+	+
Seed	+	+	-	-	-	-	+	+	-
Leaf	+	+	+	-	-	-	+	+	-
Bark	+	+	-	+	-	+	-	+	+
Root	-	-	-	+	-	-	-	-	-

+ = presence; - = absence of bioactive compounds.

1979).

The fruit extract has been shown to be used as antimicrobial agent against gram positive and gram negative bacteria (Tatsadjieu et al., 2004). *X. aethiopica* has anti-spirochoectal properties so that it works both as a preventive measure and in treatment of primary, secondary and tertiary stages of syphilis. *X. aethiopica* has been used for treating rheumatism as well as other inflammatory conditions (Tatsadjieu et al., 2004).

Phytochemicals as antioxidants play vital roles in human health (Ivan, 2003; Ayoola et al., 2008; Yusuf et al., 2014). *X. aethiopica* has been found to contain some phytochemicals which exhibit a wide range of biological effects as a consequence of their antioxidant properties (Fleischer, 2003; Keita et al., 2003). The chemical components of *X. aethiopica* have been helpful in the avoidance and treatment of cancerous tumors.

An alkaloid is a type of plant derived organic compound. Alkaloids are generally composed of oxygen, hydrogen, carbon and nitrogen. Some alkaloids are considered toxic but others are often used medicinally (Ivan, 2003; Yusuf et al., 2014). Many alkaloids can be used for medical purposes (Fleischer, 2003; Keita et al., 2003). Atropine for instance is used to stimulate the central nervous system and to dilate the pupils of the eyes (Keita et al., 2003). Anonecaine, an alkaloids constituent of *X. aethiopica,* is known to have anti-pyretic effect. They are powerful antibiotics and valuable medicine against malaria as well as their application in local anesthesia as pain relief (Kinghom and Baladrin, 1993; Bruneton, 1999; Harbone, 1998)

Flavonoids represent the most common and widely distributed of plant phenolics found in *X. aethiopica*. Flavonoids prevent oxidative cell damage, have strong anti-cancer activity and protects against all stages of carcinogenesis (Keita et al., 2003; Yusuf et al., 2014; Aguoru et al., 2014). As antioxidants, flavonoids from *X. aethiopica* provide anti-inflammatory action. Flavonoids are important antioxidants and promote several health effects. Aside from antioxidant activity, these molecules provides the following beneficial effects; antiviral, anti-cancer, anti-inflammatory and anti-allergic (Fleischer, 2003).

Saponins another phytochemical constituent of *X. aethiopica* have wide range of biological properties, they are used to recover homeostasis, have anti- inflammatory and anti- cancer actions. Saponins cause a reduction of blood cholesterol by preventing its re-absorption. They have antitumor and antimutagenic activities and can lower the risk of human cancers by preventing cancer cells from growing apart from their biocidal effects against pathogens (Morisaki et al., 1995; Fleischer, 2003; Evans, 2003; Yusuf et al., 2014; Aguoru et al., 2014).

The roles of active principles, of plant origin, in drug synthesis cannot be over emphasized. Therefore, this work aimed at investigating the types and composition of phytochemicals of medicinal importance contained in the petiole, leaf, seed, stem bark and root of *X. aethiopica* in North Central Nigeria, tropical West Africa.

MATERIALS AND METHODS

Plant parts (petiole, leaf, seed, stem bark, and roots) were collected from Vandeikya Local Government Area of Benue State, North Central Nigeria. The extraction method of Harborne (1973) was employed. Extracts were qualitatively screened for phytochemicals of medicinal importance following standard protocols (Sofowara, 1986; Aguoru et al., 2014). This was followed by quantitative analysis of bioactive ingredient common to all parts of the plant using double extraction gravimetric method. Data were statistically analysed using SPSS software for paired correlation and test of significance of the phytochemicals at 95% confidence limit.

RESULTS AND DISCUSSION

The phytochemical compositions of *X. aethiopica* tested are summarized in Table 1. The results revealed the presence of medically active compounds in all the parts studied. Alkaloids, saponins, tannins, reducing sugar, phlobatannins, anthraquinones, steroids, flavonoids, and glycosides were present in all the petiole, seed, leaf, bark and root of the plant. Comparatively, reducing sugar and phlobatannins were absent in the petiole while tannin, reducing sugar, phlobatannins and anthraquinones, and glycosides were absent in the seed and leaf. Tannins, phlobatannins and steroids were absent in the stem bark while the root contained only reducing sugars.

The quantitative estimation of the chemical is presented

Table 2. Percentage active principles in plant parts.

Plant part	Saponin	Flavonoid	Alkaloid (%)
Stem bark	8.33	5.24	5.67
Seed	2.56	2.03	2.43
Petiole	0.33	1.21	1.43

Table 3. Paired sample correlations of the phytochemical.

Sample		N	Correlation	Significance, P(0.05)
Pair 1	Saponin and flavonoid	3	0.997	0.051
Pair 2	Saponin and alkaloid	3	0.999	0.029
Pair 3	Flavonoid and alkaloid	3	0.999	0.022

Table 4. Paired samples test of significance.

Sample	Paired differences					t	df	Sig. (2-tailed)
	Mean	Std. deviation	Std. error mean	95% Confidence interval of the difference				
				Lower	Upper			
Pair 1 Saponin - flavonoid	0.91333	2.01257	1.16196	-4.08616	5.91283	0.786	2	0.514
Pair 2 Saponin - alkaloid	0.56333	1.91709	1.10683	4.19898	5.32565	0.509	2	0.661
Pair 3 Flavonoid - alkaloid	0.35000	0.11358	0.06557	-.63214	-.06786	5.337	2	0.033

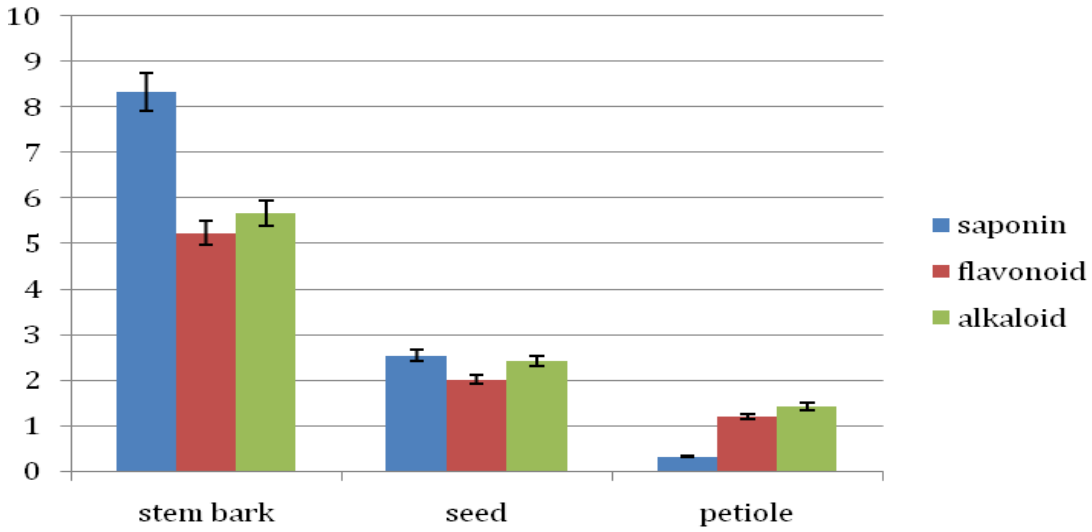

Figure 1. Plant parts and their compositions with standard error.

in Table 2. The stem bark contained very high amount of saponin (8.33%), alkaloid (5.67%) and flavonoid (5.24%). The seed had moderate amount of the three phytochemicals which were relatively low in the petiole. It has been revealed that saponin is positively correlated with alkaloid (+0.999) at 0.029 level of significance. The high positive correlation between flavonoid and alkaloid (+0.999) is the most significant value (p=0.022) (Table 3). This indicates that the presence of flavonoid in any part of this plant is accompanied with alkaloid and *vice versa*. Saponin and flavonoid are also positively correlated but statistically insignificant. Generally, the high amount of flavonoid and alkaloid is statistically significant (p=0.033) in the three plant parts quantified (Table 4 and Figure 1)

In terms of phytochemical quantity, the stem bark is the most important part of medicinal importance followed by the seed while petiole is the least valuable of the three (1). Therefore, saponin, flavonoid and alkaloid are highly

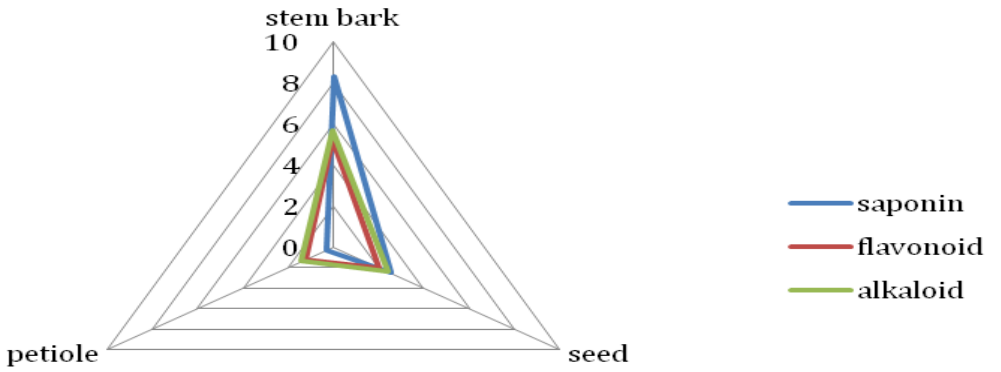

Figure 2. Region of saponin, flavonoid and alkaloid concentration in plant parts.

concentrated in the stem bark (Figure 2). Based on functional types of phytochemicals, the petiole is the most important part which contains 7 out of 9 active principles screened.

The high amount of flavonoid in the stem bark, petiole and seed supports the anti-inflammatory, antimicrobial and antitumor activities of *X. aethiopica* reported by Fleischer (2003). The high amount of saponnin, alkaloid observed in this study therefore accounts for their bitter taste and confirms their numerous therapeutic functions (Harbone, 1973).

Reports on the medicinal uses of *X. aethiopica* by many authors across the globe (Karawya et al., 1979; Fleischer, 2003; Keita et al., 2003; Asekun and Adeniyi, 2004) are fully supported by this work. This is because the plant contains significant amounts of major active principles of therapeutic benefits. The correlations among the quantified phytochemicals therefore suggest the multifaceted uses of this plant in curing many types of diseases and ailments.

In conclusion, it has also revealed all parts of the plant such as petiole, leaf, seed, stem bark and root are of medicinal importance. Therefore, the tree could be used as a source of making cheap drugs of diverse curative values. It is suggested that further work should be carried out to isolate and purify the active constituents reported. The activity of the various active principles should also be tested on microorganisms and laboratory animals.

Conflict of Interests

The authors have not declared any conflict of interests.

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