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Full Length Research Paper

Biological activities and phytoconstituents of the lower plant *Platycerium angolense,* Welwex Hook

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Lower plants are regarded as unstudied portion of plant kingdom that are not traditionally employed for healing, treatment and management of ailments in folklore medicine. The ethanolic extract of *Platycerium angolense,* Welwex Hook was phytochemically screened and analyzed for flavonoid content with a view to investigating its biological activities. The flavonoid fractions were assayed for anti-oxidant, 1, 1, diphenyl-2-pycrilhydrazyl (DPPH) free radical scavenging, anti-inflammatory, inhibition of free radical-induced haemolysis and red blood cell membrane stabilizing activities and the toxicological effects on some biochemical parameters in white albino rats. The results of the present study revealed that phytoconstituents of *P. angolense* included: saponins, anthraquinones, flavonoids, tannins, deoxysugars and cardiac glycosides. The flavonoid fractions possessed and exhibited appreciable free radical scavenging, anti-oxidant and anti-inflammatory activities. The fractions also inhibited appreciably free-radical induced haemolysis of red blood cells and exerted membrane stability on bovine erythrocytes exposed to both heat and hypotonic- induced lyses. The activities of the fractions were concentrations dependent and compared favourably with those of standard drugs acetaminophen, prednisolone, rutin, and ascorbic acid. Toxicologically, the fractions caused hepatopathy and metabolic derangement in the treated rats. Based on the phytochemicals and biological activities exhibited by the extract of *P. angolense* and its flavonoid fractions, we conclude that the plant contained potentially useful bioactive molecules with therapeutic activities, and can therefore, be listed as a medicinal plant. However, care must be exercised when the plant is to be used for any form of treatment.

Key words: *Platycerium angolense,* epiphytes, phytoconstituents, erythrocytes, free-radicals, haemolysis, hepatocytes, scavenging activity.

INTRODUCTION

Platycerium are epiphytes, growing naturally on branches and trunks of trees in the tropical, subtropical jungles and rain forest of Southeast Asia, Phillippines, Indonesia, Australia, New Guinea, Africa and South America. They grow in overlapping layers with serrated or scalloped upper edges and stand erect forming an opened receptacle for water, dead leaves and organic debris that eventually decay and provide nutrients for the ferns (Huntington, 2007; Benzing, 1990; Bieding and Fischer, 1996). In Figure 1 is the photograph of *Platycerium angolense*, Welwex Hook on the host tree.

Though, there is general awareness on the utilization of botanicals in healthcare systems all over the World (Farnsworth, 1990; Hoareau and Da-Silva, 1999), there is dearth of scientific information on the phytoconstituents, potentials and therapeutic uses of lower plants (Balik, 1990). Unlike many rainforest plant species, lower plants are not normally employed by indigenous people for healing, treatment and management of ailments (Cox, 1990; Debrovner, 1992). As such, this study was designed to investigate the nature of phytoconstituents of *P. angolense* and biological activities of its flavonoid fractions.

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Figure 1. Photograph of growing *P. angolense* on the trunk of host plant.

MATERIALS AND METHODS

Plant materials

Collection and identification of plant materials

Fresh leaves of *P. angolense*, Welwex Hook, were collected from the trunks of the host tree (*Azadiractha indica*, A. Juss, Neem) opposite Staff Club and beside H. O. Library, Obafemi Awolowo University, Ile-Ife, Nigeria. The plant was identified and authenticated on Dr. F. A. Oloyede, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The leaves were cut into tiny bits, washed, air-dried in the laboratory over a period of about two months and ground into powder using manual grinding machine.

Reagents and chemicals

All the reagents used in the study were of analytical grade. They were obtained from various sources such as British Drug House Limited (BDH) London, Sigma Fine Chemicals Limited, Upsalla, Sweden, Fluka Chemical Company Plc. Germany. Reagent kits to assay for Alanine / Aspartate aminotransferase (AST/ALT) were obtained from Randox Laboratories Ltd, United Kingdom. All solutions, buffers and reagents were prepared with glass-distilled water and stored in the refrigerator.

Animals

Healthy white albino rats (20) with an average weight of (187.27 ± 5.82) g were obtained from the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were acclimatized for two months in the laboratory where they were fed with Standard Mouse Cubes (Ladokun Feeds Limited, Ibadan) and watered *ad libitum.* The rats were randomly grouped into three of five rats each.

Spectrophotometric readings

The absorbance readings were recorded on both Cam-Spec Vis-Spectrophotometer and Pharmacia Biotech Novaspec II Spectrophotometer.

Centrifugation

All centrifugation procedures were carried out on Bench Centrifuge Model 800D (Microfield Instrument England, UK.). Preparation of blood plasma, erythrocytes and liver homogenates was carried out as earlier reported (Oyedapo et al., 2004; Olagunju et al., 2000).

Methods

Preparation and fractionation of ethanolic extract of *P. angolense*

Ethanolic extract of the leaves of *P. angolense* was prepared according to the procedure as earlier described by Oyedapo and Amos, (1997) to afford a brown residue. The extract was separated by Silica-gel column chromatography using dichloromethane: ethylacetate mixtures 4:1, 3:2, 2:1 and dichloromethane: ethylacetate: methanol mixture 1:2:1 as described by Lin et al. (2008) to afford fractions A, B, C, D, E, and F. Fractions E and F were identified by spectroscopic methods and chemical reactions to be flavonoid mixtures.

Phytochemical screening

The ethanolic extract was subjected to phytochemical screening for the presence of secondary metabolites using standard procedures earlier described by Sofowora (2006), and Evans (2002).The extract was screened for the presence of alkaloids, saponins, anthraquinone, tannins, cardiac glycosides, triterpenes and flavonoids.

Estimation of flavonoids concentration

The concentration of flavonoids in the ethanolic extract as well as in the fractions was estimated spectrophotometrically according to the procedure of Sun et al. (1999). The concentration of flavonoids was expressed as rutin equivalent according to the expression below:

$$\text{Concentration of flavonoids} = \frac{\text{Abs of test} \quad \text{X conc. of standard}}{\text{Abs of standard}}$$

where abs test = absorbance of test, Abs standard = absorbance of standard, Conc. = concentration.

Estimation of total phenol

The total phenol in the various fractions was estimated according to a procedure that was based on the method described by Singleton et al. (1999). The values were expressed as tannic acid equivalent.

Biochemical analyses

Induction and inhibition of free radical-induced oxidative haemolysis of red blood cells

The induction of haemolysis was carried out by using a modified method of Amzal et al. (2008) using freshly prepared 2% (v/v) red blood cell (1.0 ml) with H_2O_2 (30%v/v, 1.0 ml) and 100 µM $CuSO_4$ (1.0 ml). The inhibition assay mixture consisted of 2% (v/v) bovine erythrocytes (1.0 ml), 100 µM $CuSO_4.5H_2O$ (1.0 ml) and H_2O_2 (30% v/v, 1.0 ml) and 1.0 ml of varying concentrations of fractions E and F. Also, rutin and acetaminophen were included in the assay. The absorbance of released haemoglobin was read at 540 nm against the reagent blank.

Assay of DPPH free-radical scavenging activity of flavonoids

The assay of 1, 1, diphenyl-2-pycrilhydrazyl (DPPH) free-radical scavenging activities of the fractions were carried out using the modified method of Blois (1985) as reported by Cakir et al. (2003) with ascorbic acid and rutin as standards. The absorbance was read at 517 nm against a DPPH control containing 1.0 ml of methanol in place of extract. Then the percentage scavenging activity was determined using the expression:

$$\%\text{scavenging activity} = \frac{(\text{Abs control - Abs sample})}{\text{Abs control}} \text{ X } 100$$

Membrane stabilizing activity assay

The membrane stabilizing activity assay method was based on the procedure described by Oyedapo et al. (2004). The assay mixture consisted of hyposaline (2 ml), 0.1 M phosphate buffer, pH 7.4 (1.0 ml), varying volumes of fractions (0.0 - 1.0 ml) at a concentration of 2.5 mg/ml and 2% (v/v, 0.5 ml) erythrocyte suspension to a total volume of 4.5 ml. The control was prepared as above without the drug while the drug control (4.5 ml) lacked erythrocyte suspension. The standard anti-inflammatory drugs for the assay were acetaminophen (1.0 mg/ml) and predinosolone (1.5 mg/ml). The reaction mixtures were incubated at 56℃ for 30 min. The absorbance of the released haemoglobin was read at 560 nm against reagent blank. The percentage membrane stability was estimated using the expression:

$$\%\text{ membrane stability} = \frac{100 - (\text{Abs test drug- Abs drug control}) \text{ X } 100}{\text{Abs. of blood control}}$$

Estimation of toxicity levels

The toxicity of fractions (E and F) was carried out as reported by Adeoye and Oyedapo (2004). White albino rats (60, 5 rats /group) were given orally fractions E and F 0.0, 25.0, 50.0, 75.0, 100.0, 125.0, and 150.0 mg/kg/bwt in a mixture of ethylacetate/water (1:1 v/v). The control group received the vehicle 1.0 ml/kg/bwt. All the rats were kept under close observation for 5 days. The minimum and maximum concentrations that produced zero percent and hundred percent deaths were obtained. Then, LD_{50} was estimated from the plot of percentage mortality versus concentration.

Biological assays

Treatment of animals

A total of fifteen white albino rats were divided into three groups of five animals each and treated as follows:

Group 1 (Control): Received normal saline at the dose of 1 ml /kg bwt per day for five consecutive days.
Group 2: Received 100 mg/kg/bwt of fraction E per day for five consecutive days.
Group 3: Received 100 mg/kg/bwt of fraction F per day for five consecutive days. The mode of administration was orally as described by Chang et al. (1992) and Waynforth (1969).

On the 6th day, animals were anesthesia with chloroform, cut open and blood was collected by cardiac puncture into an anticoagulant. Livers were also aseptically removed, washed free of blood, dried on tissue paper, wrapped with aluminum foil and then kept in deep freezer for further analyses.

Biochemical assays were carried out on the plasma and liver homogenates to estimate total protein (Schacterk and Pollack, 1973), albumin, globulin and pyruvate concentrations (Chawla, 1999), alkaline phosphatase and acid phosphatase (Oyedapo 1996), alanine aminotransferase (ALT) and aspartate aminotranferase (AST) (Reitman and Frankel, 1957) assays were performed using Randox Diagnostic kits (Randox Laboratories Ltd, Crumlin, UK).

Statistical analysis

Data and biochemical parameters were expressed as the mean ± SEM (standard error of mean) and subjected to the Student's't' test. The significant of the difference between the control and treated rats were tested at 5% level of probability (Rao and Richard, 2007).

RESULTS

The ethanol extract gave positive test reactions for the

Table 1. Percentage DPPH-radical scavenging activities of rutin, ascorbic acid and fractions E and F.

Compound	Concentration (mg/ml)	Percentage scavenging activity
Fraction E	0.1	85.78 ± 2.09
	0.01	66.98 ± 4.08
	0.001	62.78 ± 2.95
Fraction F	0.1	79.74 ± 3.47
	0.01	68.07 ± 1.04
	0.001	53.55 ± 5.19
Rutin	0.1	88.62 ± 2.31
	0.01	50.38 ± 4.38
	0.001	45.14 ± 3.18
Ascorbic acid	0.1	95.44 ± 2.74
	0.01	91.93 ± 1.68
	0.001	36.36 ± 1.54

Each value represents the mean ± SEM of n=9 readings.

presence of saponins, anthraquinone, flavonoids, tannins, deoxysugars and cardiac glycosides. The cumulative yield of each fraction was A (1.52 g); B (0.65 g); C (2.21 g); D (2.45 g); E (6.21 g) and F (5.25 g). The flavonoid concentrations of the crude, ethanol extract and fractions E and F were 2.33 ± 0.02 and 1.23 ± 0.07 mg/ml and 1.84 ± 0.08 mg/ml, respectively as rutin equivalent.

Total phenol contents of the fractions of *P. angolense* using methods of Singleton et al. (1999) gave the mean values of 4.75 ± 0.13 and 6.65 ± 0.16 mg/g for fractions E and F. The values were expressed as tannic acid equivalent. The toxicity level was estimated to be 112.5 and 115.5 kg/bwt for fractions E and F, respectively.

DPPH free- radical scavenging activity

In Table 1 is the summary of DPPH-radical scavenging activity assay of the fractions (fractions E and F), rutin and ascorbic acid. It was noted that the scavenging activity was concentration dependent with fraction E exhibited higher activity than fraction F. The IC_{50} values were 33 and 30 µg/ml for fractions E and F respectively. The activities were slightly lower than those of rutin and ascorbic acid.

Membrane stabilizing activities of fractions E and F, acetaminophen and prednisolone on bovine red blood cells

Figure 2 (a-d), are the membranes stabilizing profiles which are exhibited by fractions E and F, acetaminophen and prednisolone on red blood cells exposed to both heat and hypotonic induced lyses. Fractions E and F exhibited monophasic modes of protection with maximum activity of 67.68 ± 1.45 and 47.14 ± 2.86%, respectively. The activities of the fractions compared favorably with those of acetaminophen (non-steroidal anti-inflammatory drug) and Prednisolone (steroidal hormone).

Inhibition of free radical induced-haemolysis of bovine erythrocytes by flavonoid fractions

Table 2 summarizes the effects of rutin, acetaminoophen, hydrogen peroxide, copper sulphate and fractions of *P. angolense* on bovine erythrocytes. In the presence of fractions and copper sulphate the red blood cells were adequately protected against free radical induced damaged.

DISCUSSION

Studies have revealed that most woody species contain tannins and many plants especially legumes and grains have high levels of tannins and phytates (esters of phytic acids). These phytochemicals (tannins and phytates) are regarded as botanical chelators and possess potential malaria suppressive effects through sequastation of iron (Etkins, 1996; Etkins and Ross, 1982). Moreover, saponins, polyphenols and phytosterols exhibit hypocholestrolemic effects on animals and humans thereby contributing to the phenomenon of the low rate (incidence) of artherosclerosis (Johns 1996; Johns and Chapman, 1995). Flavonoids on the other hand are

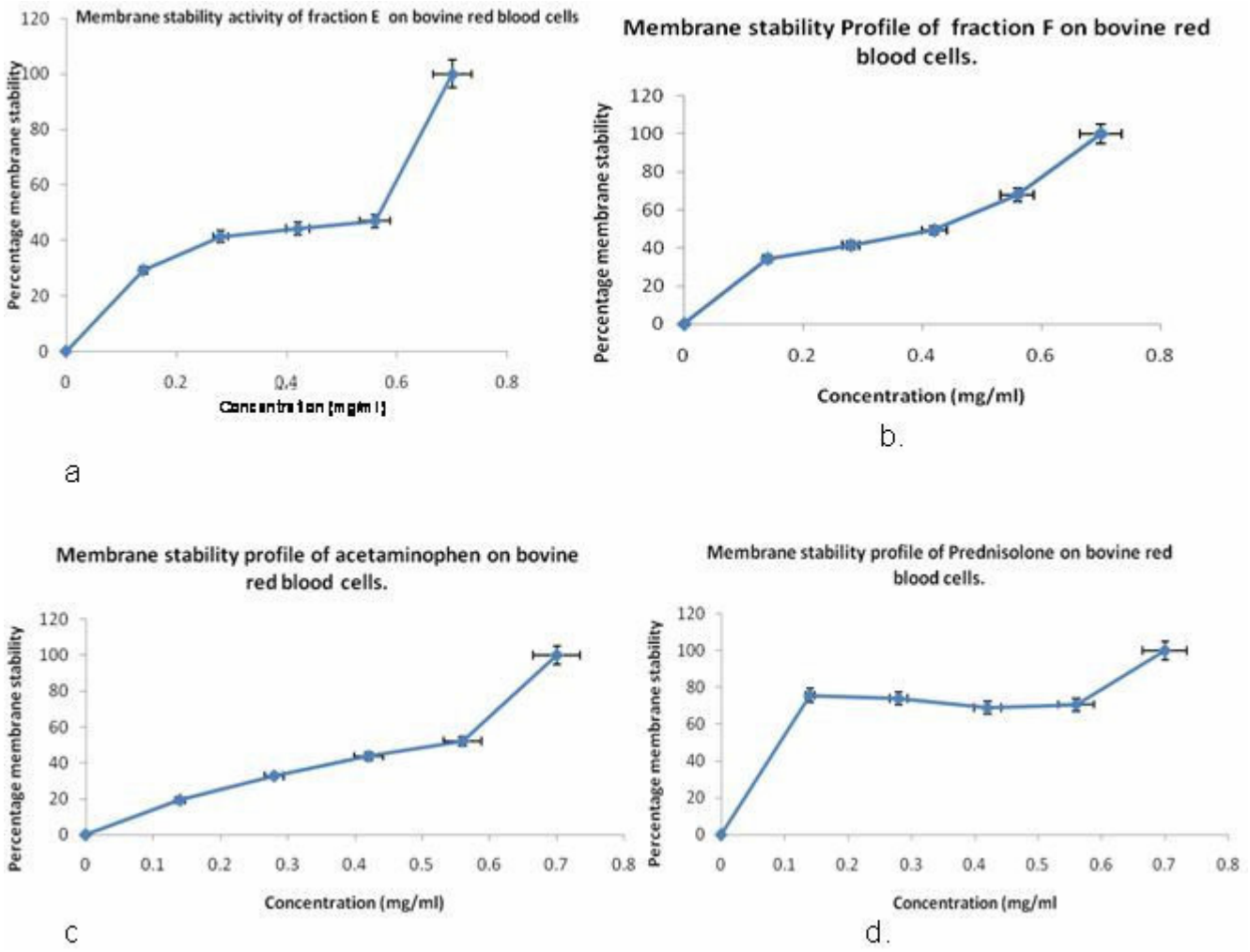

Figure 2 (a-d). Membrane stabilizing profiles of fractions E and F, acetaminophen and prednisolone on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean ± SEM of 4 readings.

Table 2. Effect of fractions E and F on free radical - induced-haemolysis.

Fraction	Percentage lysis	Percentage protection
rbc + rutin	41.83 ± 2.31	58.17 ± 1.23
rbc + acetaminophen	53.02 ± 3.21	46.98 ± 1.56
rbc + $CuSO_4$ +H_2O_2	88.65 ± 1.32	11.35 ± 1.65
E +rbc +$CuSO_4$ +H_2O_2	25.43 ± 1.22	74.57 ± 1.21
F + rbc +$CuSO_4$ +H_2O_2	22.19 ± 1.46	77.81 ± 1.12

Each value represents the mean± SEM of n=6 readings. $p \leq 0.05$ was taken as statistically significant.

actively involved in plant metabolic processes especially energy transfer, control of respiration, photosynthetic, action of growth hormones and growth regulations as well as morphogenesis, sex determination and defense against infections (Middleton and Kandaswami, 1994; Middleton, 1996).

Fractions E and F which were eluted mainly with solvent systems (CH_2Cl_2: EtOAc 2:1) and CH_2Cl_2/EtOAc /MeOH 1:2:1) gave positive test reactions for the presence of flavonoids. The results agreed with the observations of Lin et al. (2008) who eluted flavonoids with DNA strand – scission activity from *Rhus javanica* with a solvent mixture (CH_2Cl_2:EtOAc 2:1). Also ethylacetate fractions of stem and bark of *Distamonauthus bontamianus* contained phenolic compounds mainly flavonoids (Nguekefack et al., 2005).

Table 3. Effect of Fractions E and F on rats blood plasma biochemical parameters.

Parameters	Group I (Control)	Group II (fraction E treated rats)	Group III (fraction F treated rats)
Total Protein (g/dL)	6.67 ± 0.29[a]	8.24 ± 0.49[b]	9.27 ± 0.33[b]
Albumin (g/dL)	4.95 ± 0.67[a]	6.95 ± 0.16[b]	6.21 ± 0.15[b]
Creatinine (mg/dL)	10.46 ± 0.88[a]	11.12 ± 1.39[a]	18.53 ± 7.21[b]
Pyruvate (mg %)	0.42 ± 0.07[a]	0.22 ± 0.04[b]	0.62 ± 0.10[b]
AciPase (µ mole/ml)	126.31 ± 8.50[a]	121.15 ± 9.13[a]	67.77 ± 1.94[b]
Alkpase (µ mole/ml).	33.44 ± 1.83[a]	20.11 ± 0.73[b]	9.25 ± 1.78[b]
AST(U/I)	27.00 ± 0.06[a]	34.11 ± 0.68[a]	14.22 ± 0.62[b]
ALT(U/I)	18.83 ± 0.57[a]	38.67 ± 0.67[b]	44.17 ± 0.08[b]

Each value represented mean±SEM) of n= 9 readings. Acid phospatase (AciPase), Alkaline phosphatase (AlkPase). The activity of phosphatases were expressed as µmole- p-nitrophenol/ml), Alanine aminotrasferase (ALT), Aspartate aminotrasferase (AST). Data shown with different letters are significantly different at the $p \leq 0.05$ level.

The results were further confirmed by the colour reactions with specific reagents for the detection of phenolics in chromatograms (1% Alcoholic $AlCl_3$ and Naturstoff reagents).

Phenols are very important phytochemicals because of their ability to scavenge free radicals owing to their redox properties which permit them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice – Evans et al., 1996). It was therefore not surprising that both fractions exerted anti- inflammatory and anti-oxidant activities. The anti-oxidant activities exerted by the extracts doubtlessly might be due to phenolic content. The phenolic compounds are directly involved in anti-oxidant action (Diplock 1997; Hatano et al., 1989).

The DPPH free radical scavenging activities of the fractions E and F, rutin and ascorbic acid are represented in Table 3. It was noted that each fraction exhibited free radical scavenging activities with fraction E exhibited higher activity. It was noted further that, the radical scavenging activity of these fractions was slightly lower than that of reference anti-oxidant compounds (ascorbic acid and rutin) but was quite comparable considering the concentration of the extract employed in this study. The radical scavenging activity was also concentration dependent. Studies have shown that the reactive oxygen species of low reactivity can be converted to a highly reactive species (De Groot and Rauen, 1998). Reaction of hydrogen peroxide (H_2O_2) with low valent forms of the transition metal ions iron (Fe^{2+}) and Copper(Cu^{2+}) ion lead to the formation of $^{\cdot}OH$ (Fenton reaction) or species of comparable reactivity such as Fe^{2+} (Ferryl ion) or $Cu(OH)_2^{+}$ a copper III complex. The hydroxyl radical $^{\cdot}OH$, abundant under physiological conditions are quite reactive, reacts rapidly with any type of biological molecules in living cells, such as sugars, amino acids, phospholipids and nucleobases (the components of nucleic acids) (Amzal et al., 2008).

Treatment of bovine erythrocytes with hydrogen peroxide in the presence of CuSO4 (Cu2+, H2O2) resulted in the increase of release of haemoglobin. However, in the presence of hydrogen peroxide, copper sulphate and fractions E and F, there was marked reduction in the amount of haemoglobin released. The fractions were able to inhibit the action of free radicals generated as a result of the reactions of $CuSO_4$ and H_2O_2. It was noted that fractions E and F gave higher inhibition and protections. The results are in agreement with those of Amzal et al. (2008) and Liu et al. (2002) that certain plant metabolites protect human erythrocytes from free radical-induced haemolysis.

Plants derived drugs have been demonstrated to contain principles that possess ability to facilitate the stability of biological membranes when exposed to induced lyses (Sadique et al., 1989; Oyedapo et al., 2004). Anti-inflammatory agents exert their effects through a variety of mechanisms including inhibition of cotton pellet granulation, uncoupling of oxidative phosphorylation, stimulation and inactivation of adenosine triphosphates and phosphatases (acid and alkaline) as well as stabilization of red blood cells exposed to hypotonic induced lyses (Sadique et al., 1989; Pal and Chandhauri, 1992; Oyedapo and Famurewa, 1995; Oyedapo, 1996; Oyedapo et al., 2004). The results revealed that fractions E and F exerted maximum membrane stability of 67.86 ± 1.45% (IC_{50} =0.11 mg/ml) and 47.14 ± 2.56% (IC_{50} =0.13 mg/ml) on bovine erythrocytes exposed to both heat and hypotonic induced lyses. The activities of the fractions compared favorably with that of acetaminophen (a non-steroidal anti-inflammatory drug) which exerted maximum percentage of 67.86 ± 1.43 (IC_{50}=0.11 mg/ml) and Prednisolone (a steroidal hormone) exerted maximum membrane stability of 70.60 ± 4.20% (IC_{50}= 0.13 mg/ml) (Table 4). Results of preliminary studies revealed that both aqueous and ethanolic extracts of *P. angolense* exerted maximum membrane stabilities of 78.91 ± 0.95 and 88.25 ± 1.12% and were extremely toxic at higher concentrations. The activity was noted to be higher than

Table 4. Effects of Fractions E and F on the liver homogenates biochemical parameters.

Parameters	Group I (control)	Group II (fraction E treated rats)	Group III (fraction F treated rats)
Total Protein (g/dL)	4.35 ± 0.05[a]	4.46 ± 0.06[a]	4.36 ± 0.07[a]
Albumin (mg/dL)	2.45 ± 0.06[a]	2.68 ± 0.04[a]	2.56 ± 0.06[a]
Creatinine (mg/dL)	38.47 ± 6.55[a]	28.62 ± 8.62[b]	51.48 ± 3.66[b]
Pyruvate (mg %)	0.19 ± 0.03[a]	0.20 ± 0.02[a]	0.22 ± 0.01[a]
AciPase (µ mole/ml)	33.44 ± 1.36[a]	39.90 ± 2.03[b]	104.16 ± 0.72[b]
AlkPase (µ mole/ml)	86.39 ± 2.37[a]	59.73 ± 2.03[b]	52.86 ± 2.30[b]
AST (U/l)	52.50 ± 2.74[a]	58.25 ± 3.10[b]	56.67 ± 2.40[b]
ALT (U/l)	72.17 ± 1.02[a]	75.67 ± 1.04[a]	69.83 ± 2.63[a]

Each value represented the mean ± SEM of n=9 readings. Acid phosphatase (AciPase), Alkaline phosphatase (AlkPase), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). The activities of the phosphatases are expressed as µmole-p-nitrophenol/ml. Data shown with different letters are significantly different at the level of $p \leq 0.05$ level.

what was observed in this present study. This could be due to the contributory effect of other phytochemicals in the aqueous and ethanolic extracts.

Quite a number of extracts have been demonstrated to protect and stabilize red blood cells that were exposed to a combined hypotonic and heat induced stress. The roots of *Threobroma cacao* (Oyedapo et al., 2004; Falade et al., 2005) exerted very high membrane stability when compared with that of *Olax subscorpioides* (87.5%), *Aspilia africana* (85%), *Fagara zanthoxyloides* (83.8%) (Oyedapo and Famurewa, 1995).

Liver is the major component in the body defenses against toxic agents due to its detoxifying ability, its excretory role and the fact that most injected or exogenous materials pass through the liver before entering the general circulation (Hayes, 1989). Liver damage could be confirmed by changes in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). High levels of transaminases indicate hepatopathy (Zilva and Pannall, 1975). The determination of the activities of alkaline phosphatase and acid phosphatase is an indication of metabolic changes under stressful conditions (Sadhu et al., 1985, Oruc and Uner 1999). High AST activities in the serum are always due to myocardial infarction, various types of liver diseases and sometimes with renal diseases, while ALT increases in the serum in infectious hepatitis. In fact, AST is employed in the diagnosis of myocardial infarction while ALT is useful in diagnosis of liver damage (Coodley, 1970).

In this study, the plasma ALT activities increased in groups II and III treated rats by 105.4 and 134.6% respectively after treatment with fractions E and F (Table 3). However, there was an increase in activity by 33.1% in AST in group II rats and a decrease of 47.3% in the activity in group III treated rats. Moreover, in the liver of the treated rats the two fractions caused increase in activities of AST by 11.0 and 7.9%, respectively. The ALT activity increased by ($p \leq 0.05$) 4.9% in group II rats and a decrease of 3.2% in group III treated rats. It could be hypothesized that fractions E and F caused hepatic metabolic derangement in the treated rats. The level of activity however, depends on the nature of the phytoconstituents in each fraction. Sadhu et al. (1985) and Olagunju et al. (2000, 2004) reported elevation of AST/ALT activities in the sera of *Channa striatus* (BL) and rats treated with paraquat and extracts of *Plumbago zylanica* and *H. madagascariensis*.

It was noted further that fractions E and F caused significant ($p \leq 0.05$) alterations in alkaline and acid phosphatase activities. In the plasma of group II rats, there was a slight increase in the activity of the acid phosphatase (Acipase) by 4.1% while the activity was significantly ($p \leq 0.05$) decreased in group III rats (46.3%). The alkaline phosphatase activities were significantly ($p \leq 0.05$) inhibited in the plasma of groups II and III treated rats by 39.9 and 72.3%, respectively. In the liver however, the Acipase activities in groups II and III treated rats increased by 19.35 and 211.5% while alkaline phosphatase (AlkPase) activities were significantly reduced by 30.9 and 38.8% in the treated groups II and III.

The results revealed that both fractions E and F exerted strong effects on the activities of AciPase and AlkPase which implied that the two fractions might have caused destruction of cell membranes and lysosomes which librated these hydrolases into the cell system of tissues to facilitate inactivation of the enzymes.

The plasma total proteins in the treated rats increased slightly but not significantly. In group II treated rats; there was an increase of 23.5% in the plasma protein level and 39.9% increase in group III treated rats. The liver total protein levels in both control and treated rats were not affected by the fractions. Albumin concentrations in both the plasma and their liver homogenates were not adversely affected which implied that transportation of materials was not impaired in the treated rats.

Earlier studies reported increase in hepatic total protein in the liver of rats treated with extract of *Momordica charantia* (Oyedapo and Araba, 2001) and Nivalenol

elevated blood total protein and glucose in egg laying hens (Garalevicine et al., 2002). Contrariwise, Olagunju et al. (2000, 2004) reported treatment of rats with the leaf and stem-bark extracts of *O. subscorpioides* and *H. madagascarensis* resulted in 71.85 and 63.47% reduction in the protein levels of homogenates of liver and kidney, while the stem-bark extract caused 69.85 and 68.12% reductions in the protein contents of homogenates of the two organs.

Treatments of rats with fractions E resulted in the drastic reduction in the plasma pyruvate level by 47.6% while fraction F resulted in the increase of pyruvate by the same margin. On the other hand, the treatment with the fractions did not alter the pyruvate levels in the liver homogenates of control and treated rats. Pyruvate is one of the central metabolites through which carbon sources are directed to immediate energy productions (the citric acid cycle) or to energy storage (gluconeogenesis or lipogenesis via the formation of acetyl-ScoA). Alternatively, it may provide the substrate for lactate dehydrogenase in a reaction that maintains NAD+ concentration in the cytoplasm (Dow et al., 1997).

Creatinine is a useful diagnostic marker, the amount excreted in a 24 h period is proportional to the muscle mass of each individual. The results showed that the two fractions might be promoting the formation of creatinine in the liver from where it is transported to both muscle and brain cells. In the plasma, there was no difference in the creatinine concentrations between the control and group II treated rats. However, the concentration in the plasma of group II rats was slightly higher but not significant. Increase in the level of plasma creatinine could not be attributed to impairment in renal metabolism because there was a concomitant increase in the concentration of creatinine in the liver of group III treated rats.

In conclusion, extract of *P. angolense* contains potentially useful phytochemicals and exhibits remarkable therapeutic activities. Therefore, lower plants like their higher plants counter-parts can, once proven, be listed as medicinal plants and employed in the management and treatment of ailments and disorders.

REFERENCES

Adeoye BA, Oyedapo OO (2004). Toxicity of *Erythrophleum guineense* stem-bark: Role of Alkalidal fraction. Afr. J. CAM, 1: 45-54.

Amzal H, Alaoni K, Tok S, Errachidi A, Charof R, Cherrah Y, Benjouad A (2008). Protective effect of Saponins from *Argania spinosa* against free radical induced oxidative haemolysis. Fitoterapia, 79: 337-344.

Balik MJ (1990). Ethnobotany and the identification of therapeutic agents from the rain forest. Institute of Economy Botany, The New York Botanical Gardens, Bronx, New- York 10458 USA. Ciba Foundation Symposium, 154: 22-29.

Benzing DH (1990). Vascular epiphytes: general biology and related biota. Cambridge University Press. Cambridge.

Bieding N Fischer E (1996). Epiphytic vegetation and ecology in Central African forests (Rwanda, Zaire). Ecotropica, 2: 121-142.

Blois S (1985). Anti-oxidant determination by use of stable free radicals. Nat., 29: 1199-1201.

Cakir A, Mari A, Yildinn A, Duru ME, Harmender M, Kazaz C (2003). Isolation and characterization of anti-oxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium,* L. by activity guided fractionation. J. Ethnopharmacol., 87: 73-83.

Chang LW, Yang CM, Chen CF, Dengs JF (1992). Experimental Podophyllotoxin (Bajialian) poisoning II: Effects on the liver, intestine, kidneys, pancreas and testis. Biochem. Environ. Sci., 5: 293-302.

Chawla R (1999). Biochemical tests for total proteins, albumin and pyruvate In Practical Clinical Biochemistry Methods and Interpretations, 2nd Edition JAYPEE, pp. 107-134.

Coodley EL (1970). Diagnostic Enzymology Philadelphia: Lea and Febinger, pp. 202-217.

Cox PA (1990). Ethnopharmacology and the search for new drugs. Ciba Foundation Symposium, 154: 40-45.

Debrovner D (1992). Edible Remedies. Am. Drug, 205: 36-40.

de-Groot H, Rauen U (1998). Tissue injury by reactive oxygen spies and the protective effects of flavonoids. Fundamentals Clin. Pharmacol., 12: 249-255.

Diplock AT (1997). Will the good fairies please prove to us that vitamin E lesson human degenerative disease? Free Radical Res., 27: 511-532.

Dow J, Lindsay G, Morrison J (1997). In Biochemistry: Molecules, Cells and the Body. Addison-Wesley England, pp. 241-245: 385-390.

Etkins NL (1996). Medicinal Cuisiness: Diet and Ethnopharmacology. Int. J. Pharmacog., 34: 313-326.

Etkins NL, Ross PJ (1982). Food as medicine and medicine as food: An adaptive framework for the international of plant utilization among the Hausa of Northern Nigeria. Soc. Sc. Med., 16: 1559-1573.

Evans WC (2002). Phenols and Phenolic glycosides. In Trease and Evans Pharmacognosy, Elsevier Science Limited, pp. 214-252.

Falade OS, Otumuyiwa IO, Oladipo A, Oyedapo OO, Akinpelu BA, Adewusi SRA (2005). The chemical composition and membrane stability of some herbs used in local therapy for aneamia. J. Ethnopharmacol., 102: 15-22.

Farnsworth NR (1990). The role of ethnopharmacology in drug development. In Bioactive components from Plants, Wiley, Chihester, Ciba Foundation Symposium, 150: 2-21.

Garalevicine D, Petterson H, Elwinger K (2002). Effect on health and blood plasma parameters of laying eggs by pure nivalenol in the diet. J. Anim. Physiol. Anim. Nutri. (Berlin), 86: 389-398.

Harborne JB (1988). The flavonoids: Advances in Research Since 1980. Chapman and Hall London.

Hatano VT, Edamatson R, Hiramatsu M, Mori A, Fyjita Y, Yasuhara D (1989). Effects of interaction of tannin with co-existing substances VI. Effects of Tannins and related polyphenols on Superoxide anion radical and on DPPH radical. Chem. Pharmaceut. Bull., 37: 2016-2021.

Hayes JA (1989). Systemic Responses to toxic Agents. In A guide to General Toxicology 2nd Revised Edition Krager AG. Basel Switzerland, pp. 24-61.

Hoareau L, Da SEJ (1999). Medicinal Plants: A re-emerging health aid. Electronic J. Biotechnol., 2(2): 56-70.

Huntington A (2007). *Platycerium elephantosis*- Elephant Staghhorn Fern. Albert's Greenhouse. Madagascar Tropical: A *Platycerium* Fern Nursery, Altadena, California- Los Angeles Country, USA, 626: 791-3600.

Johns T(1996). Phytochemicals as Evolutionary Mediators of Human Nutritional Physiology. Int. J. Pharmacol. (USA), 34: 327-334.

Johns T, Chapman L (1995). Phytochemicals injested in traditional diets and medicines as modulators of energy metabolism. In Phytochemistry of Medicinal Plants: Recent Advances in Phytochemistry, 29, Edited by JT Arnason R Mata, JT Romeo, Plenum press New York (NY), pp. 161-168.

Kumar PE, Sadique J (1987). The biochemical mode of action of *Gynaudronnis gynandra* in inflammation. Fitotrapia LVIII, 379-385.

Lin CN, Chen HL, Yen MH (2008). Flavonoids with DNA strand-scissions activity from *Rhus javanica* var *roxburghana*. Fitoterapia, 79: 32-36.

Liu ZQ, Luo XY, Sun YX, Chen YP, Wang ZC (2002). Can ginsenosides protect human erythrocytes against free-radical-induced haemolysis? Biochimica et Biophysica Acta, 1572: 58-66.

Middleton E Jr. (1996). Biological properties of plant flavonoids: An Overview. Int. J. Pharmacog., 34: 344-348.

Middleton EJ, Kandaswami C (1994). In “The flavonoids: Advances in Research since 1986. Edited by J.B. Harborne, Charpman and Hall, London, pp. 619-652.

Nguelefack EMP, Ngu KB, Atchade A, Dimo T, Tsabarg N, Mbefor JT (2005). Phytochemical composition and *in vitro* effect of the ethylacetate bark extract of *Distemonanthus berithaminanus* (Caesalpiniaceae) on *Staphylococcus aureus* and *Streptococcus agalactiae*. Cameroon J. Exp. Biol., 1(1): 50-53.

Olagunju JA, Oyedapo OO, Onasanya BA, Oshoba OO, Adebayo OO, Eweje O, Sodehinde AB (2000). Effects of isosaline extract of *Tetrapleura tetraptera* and *Olax subscorpioides* on certain biochemical parameters of albino rats. Pharmaceut. Biol. (USA), 38: 1-4.

Olagunju JA, Ogunfeibo AB, Ogunbosi AO, Taiwo OA (2004). Biochemical changes elicited by isosaline leaf and stem bark extracts of *Haruguana madagascarensis* in the rat. Phytother. Res. (London), 18: 588-591.

Oruc EO, Uner N (1999). Effects of 2, 4- Diamin on some parameters of protein and carbohydrate metabolisms in the serum, musle and liver of *Cyprinus carpio*. Environ. Pollut., 105: 267-272.

Oyedapo OO, Famurewa AJ (1995). Antiprotease and membrane stabilizing activities of extracts *Fagara zanthoxyloides, Olax subscorpioides* and *Tetrapleura tetraptera*. Int. J. Pharmacog., 33(1): 65-69.

Oyedapo OO (1996). Studies on the biological activities of the root extract of *Plumbago zeylanica*. Int. J. Pharmacog., 34(5): 365-369.

Oyedapo OO, Amos S (1997). Further investigations into the bioactivity of the root extract of *Plumbago zeylanica* Linn. Phytother. Res., 11: 62-63.

Oyedapo OO, Araba BG (2001). Stimulation of protein biosynthesis in rats hepatocytes by extract of *Momordica charantia*. Phytother. Res., 15: 95-98.

Oyedapo OO, Akinpelu BA, Orefuwa SO (2004). Anti-inflammatory effect of *Theobroma cacao*, L. root extract. J. Trop. Med. Plants (Malaysia), 5(2): 161-166.

Pal S, Chaudhuri NAK (1992). Further studies on the anti-inflammatory profile of the methanolic fraction of the fresh leaf of *Bryophylum pinmatum*. Fitoterapia (Italy) LXIII, pp. 457-459.

Rao SPSS, Richard J (2007). In Introduction to Biostatistic and Research Methods, Asoke K. Ghash, Prentice-Hallof India, New Delhi India, pp. 70-107.

Reitman S, Frankel S (1957). A colometric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 3: 58-65.

Rice EC, Miller NJ, Paganga G (1996). Structure and antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol. Med., 20: 933-956.

Sadhu KA, Chowdhury DK, Mukhopadhyay PK (1985). Relationship between some serum enzymes, histological features and enzymes in hepatopancreases after sublethal exposure to malathion and phosphamidon in the murrel *Channa striatus* (BL). Int. J. Environ. Stud., 24: 35-41.

Sadique J, Al RWA, Baghath MF, El GRR (1989). The bioactivity of certain medicinal plants on the stabilization of the RBC system. Fitoterapia (Italy), LXVI: 525-532.

Schacterk GL, Pollack RL (1973). A simplified method for the quantitative assay of small amount of protein in biologic material. Anal. Biochem., 51: 654-660.

Singleton VL, Orthofera R, lamuela RRM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteaus reagent. Methods in Enzymol., 299: 152-179.

Sofowora A (2006). Phytochemical screening: In “Medicinal plants and Traditional Medicine in Africa”, Second Edition, Spectrum Books Limited Ibadan, Nig., pp. 150-153.

Sun PX, Yie LK, Zhang ZL, Hu M, Lu L (1999). Colometric determination of the total content of the flavonoids in epimedium capsules. J. Shenyang Pharmaceut. Univ., 16: 68-70.

Waynforth HB (1969). Animal operation Techniques: Mouse, Rat, Guinea-pig and Rabbit. In Techniques in Protein Biosynthesis Campbell PV, Sergent JR Eds, Vol. V Academic Press, London, pp. 220-249.

Zilva JF, Pannalla PR (1975). Clinical Chemistry in Diagnosis and Treatment. Lloyd-luke London, pp. 15-50.