PHYTOCHEMICAL SCREENING AND BIOCHEMICAL EFFECTS OF SOME LOCAL MEDICINAL PLANTS PARTS ON ALBINO RATS

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ABSTRACT

The study was to evaluate the apparent safety and potential hazards of medicinal plants parts. *Psidium guajava* leaves, *Eugenia aromatica*_cloves, *Distemonanthus benthmianus*_stem bark *Zingiber officinales* fruits, *Piper guineense* berry and *Cymbopogon citratus* leaves aqueous extracts and juices were screened for alkaloids, flavonoids, tannins, glycosides-both cardiac and cyanogenic, saponins and reducing sugars. The extracts were administered orally to albino rats for a consecutive period of 14 days and 5 serum enzymes Glutamate-Oxaloacetate Transaminase, Glutamate-Pyruvate Transaminase, Alkaline Phosphatase, Acid Phosphatase and Lactase Dehydrogenase activities were evaluated. It was discovered that the plants parts were constituted of various phytochemicals, some of which have been known to have deleterious effects. However, the result of their effects on the serum enzymes did not reveal any appreciable difference between neat and extract-administered rats. ($P>0.05$) for all the enzymes studied. The implications of this was discussed with a view to forestall abuse in the use of medicinal plants (chronic administration) and to exercise restraint on the efficacy of extracts as remedy in compromised organs in pathologic conditions.

Keywords: *Phytochemical screening, Medicinal plants, Serum enzymes*

INTRODUCTION

Of the over 300,000 plant species found in the world, more than 2/3 are found in the tropical regions alone. (Hoareau, 1999) These provided abundant source of plants with medicinal properties for the locals to meet their health needs (Akindewo, 1999) However, arising from studies, the safety and efficacy of these plants have been questioned (Ernst, 1998). The way out of these debate is to undertake detailed experiment to establish the phytochemicals in these plants; using appropriate extraction methods and finding out the effects they have an laboratory animals and consequently on humans.

We looked at six apparently medicinal plants parts namely; *Psidium guajawa* leaves *Eugenia aromatica* cloves, *Distemonanthus benthmianu* stem bark *Zingiber officinale* fruits, *Piper guineense* berry and *Cymbopogan citratus* leaves. The uses of these by the locals are documented in Akindewo, (1999). There are scientific papers which established that *Psidium guajava*_has anti-diarrhoea principles (Somarriba, 1998, Lutterodt, 1989) *Eugenia aromatica* cloves was reported to elicit antibacterial principles which remedied cholera and some forms of gastric irritation and dyspepsia (Akubuo, 1986).

Distemonanthus benthmianus stem bark has been reported to have antimicrobial activity and used locally for skin diseases and preventing mouth odour (Adeninokun et. al, 1999 and Morris, 1998). *Zingiber Officinale* fruit has a plethora of documented evidences such as the relief of gastro intestinal distress, liver disease, hypertension, lung infection, nausea, stomach acids and rectal prolapse (Platel and Scrinivasan, (1999) and also in reducing symptoms of osteoarthritis of the knee. (Altman and Marcussen, 2001) *Piper guineense* has very scanty scientific proofs but the locals belief it reliefs of stomachache and influences female fertility. (Akindewo, 1999).

Cymbopogen citratus leaves extracts has been reported to be efficacious against chloroquine-resistant *Plasmodium yeoli nigeriensis* (Kimbi and Fagbenro-Beyioku, 1996). The locals use it for jaundice, as a diuretic, antidiarrhoeal, and carminative. (Akindewo, 1999). Despite the apparent effects of these plants parts, active principles have not all being fully reported, making the need for photochemical screening important.

Volatile oils have been extracted from *Cymbopogen citratus,* (Kasali et. al, 2001) *Zingiber officinale* (Yoshikawa, (1993) *Eugenia aromatica* (Wu, et. al. 1996) and tannins with vitamins A and C from *Psidium guajava* (Somarriba, 1998). Also. *Piper guineense* is been reported to have aschantine and yangambine (Platel and Srinivasan, 1999). However, very little is known about *Distemonanthus benthmianus.*

The major site of metabolism in systemic circulation is in the liver (Baron, 1984). Liver is exposed to several different xenobiotics, some of which could be poisonous. The apparent dearth of information on the detailed components of plants extracts will require that periodic checks be made on serum enzymes status during phytotherapy. In our work, we included broad range of organ markers to monitor the effects of acute oral administration on serum enzyme activities. The enzymes investigated included Serum Glutamate- Oxaloacetate Transaminase (S.G.O.T) Serum Glutamate-Pyruvate Transaminase, (S.G.P.T.) (both use to assess liver function and heart, respectively) Alkaline Phosphatase (bones), Acid Phosphate (Kidney and Prostate glands) and Lactate dehydrogenase (erythrocyte-intergrity and muscles) Carter, (1999), Baron, (1984) and Bolarin (1997).

MATERIALS AND METHOD

Chemicals and reagents

All chemicals used in this report are sourced from Sigma-Chemical Company and were of analytical grade. They include Bismuth nitrate, Potassium iodide, acetic acid, mercury chloride, lead acetate, phloroglucinol, sodium hydroxide, ferric chloride, acetic anhydride, concentrated tetraoxosulphate (vi) acid, pyridine, sodium nitropruside, 3,5 dinitrobenzoic acid, methanol, resorcinol and concentrated HCl and chloroform for phytochemical screening and anesthesia. Others: are enzyme essay reagents.

Plant Materials and aqueous extractions

Psidium guajava and *Cymbopogon citratus* leaves were collected within the premises of the Lagos State Univeristy, Ojo. The fruits of *Zingiber officinales, Piper guineenses* berry, *Eugenia aromatica* clove and *Distemonanthus benthmianus* stem barks were purchased form a local market in Lagos. The plants parts were authenticated and vouchered in the Pharmacognosy department of the College of Medicine of the University of Lagos. They were washed and dried for further processing squeous extracts of *P.guajava* and *C.citratus* leaves were prepared according to the method of Olukoya et.al (1993). 100g of dried crushed plant parts were soaked in 250ml of sterile distilled water for seven days and concentrated to a paste under reduced pressure at 50°C using a rotary evaporator (Gallenkamp UK) Extracts were further steriled by passage through a 25mm Millipore filter. Dosages of 50mg/kg was prepared by weighing and dilating in distilled water under sterile conditions. 50m Aqueous *Z. officinales* *P.guineenses*, *D.benthmianus* and *E. aromatica* were according to the method of Cole-showers and Okochi (2001). 165g of plant parts was subjected to extraction under reflux with 2.5L of distilled water at > 100°C for 6 hours in a soxhlet apparatus. Extract was concentrated to a paste under reduced pressure at 50°C as earlier described.

Phytochemical Screening of Extracts

Phytochemical screening of extracts was as described in Harborne, (1984) Phytochemicals screened for included Alkaloids, tannins, flavonoids, cardiac glycosides, cyanogenic glycosides, Anthraquinone, Saponins and reducing sugars. Three (3) types of alkaloid test were carried out (Dragendorf, Wagner and Mayer). Two (2) tests were carried out for tannins (Catechol tannins and condensed tannins), 2 tests for saponins (frothing test and emulsion

test), 3 tests for flavonoids; 5 tests for cardiac glycosides (-Lieberman's, Salkowski, Keller-kelian, Legal and Keddle tests). One test for anthraquinone (bortrager's test) and 3 tests for reducing sugars (Fehling, Phloroglucinol and resorcinol tests).

Animal studies

Fifty-two albino rats (Wistar strains) weighing between 150 and 160g (154.2±3.1g) were purchased from the animal laboratory centre of the college of medicine of the University of Lagos and sheltered in well ventilated metabolic cages. They were maintained on Pfizer rat pellets and had water *ad libitum* throughout the duration of study (15 days) under 12 hours day/night cycle in a room temperature of 26°C-28°C. The rats were divided on weight adjusted basis into 7 groups of 4,8,8,8,8,8,8 respectively for control and 6 extracts administration. The test groups were orally administered 50mg/Kg body weight daily between 9h to 11h (G.M.T) for 7 and 14 days respectively (4 rats each that is 8 rats for each extract) consecutively. The control group was administered distilled water and sacrificed on the day of commencement of experiment to obtain, basal reference values for evaluation of parameters.

Collection Of Blood Samples, Serum Preparations And Enzyme Assays

Blood samples were collected on the 1st, 8th and 15th day and serum was prepared as described by Adeola, et. al. (2001). Enzyme assays were determined as described by Bolarin (1997). The enzymes assayed for included serum glutamate-oxaloacetate transaminase, (SGOT), serum glutamate-pyruvate transaminase (SGPT), serum alkaline phosphatase (SAKP), serum acid phosphatase(SCAP) and serum lactate dehydrogenase (SLD). Total and direct bilirubin concentrations were also determined.

Stastistical Analysis

The parameters obtained were measured as mean ± standard deviation of 4 values. Differences between mean values were analysed using the student t - test. P values less than 0.05 was considered significant while P values greater than 0.05 was interpreted as insignificant.

RESULTS

TABLE 1: Phytochemical Screening Of Aqueous Medicinal Plants Extracts

Phytochemicals	*P. guajava*	*E.aromatica*	*D.benthmianus*	*Z. officinales*	*C. citratus*	*P.guineense*
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Cyanogenic glycosides	-	-	-	-	-	-
Anthraquinone	-	-	+	+	+	-
Saponins	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+

(+) present, (-) absent

The result above summarizes the major findings of our screening. However, the result is not as simple as it appears. The undisplayed details for each extract is that *P.guajava, E.aromatica, P.guineense* tested negative for Mayer's, lieberman's, borntragers and phlobatanins, they are thus, very comparable. All extracts were negative for cyanogenic glycosides.

Table 2: Effects Of Extracts On Sgot

	DAY 7		DAY 14	
Aqueous Extracts	Mean+S.D*	P value	Mean+S.D*	P value
P.guajava	*85.80±0.25*	*1.60*	*83.60±1.23*	*0.383*
E.aromatica	*85.20±0.95*	*0.88*	*83.50±3.40*	*0.339*
D.benthmianus	*86.60±0.33*	*3.52*	*86.20±1.41*	*1.042*
Z.officinales	*84.80±0.24*	*0.57*	*85.50±1.19*	*0.755*
C.citratus	*86.50±0.98*	*1.54*	*83.70±1.28*	*0.315*
P.guineense	*86.53±0.87*	*2.25*	*83.50±3.47*	*0.182*

Control value= 84.25±0.80 IU/L

*= Mean±S.D values for 4 rats.

Table 3: Effects Of Extracts On Sgpt

	DAY 7		DAY 14	
Aqueous Extracts	Mean+S.D*	P value	Mean+S.D*	P value
P.guajava	*66.20±0.95*	*0.982*	*67.50±0.75*	*0.633*
E.aromatica	*67.40±0.66*	*1.169*	*65.50±1.17*	*2.132*
D.benthmianus	*66.70±0.51*	*2.116*	*66.80±0.40*	*0.323*
Z.officinales	*68.60±0.84*	*1.699*	*67.55±1.19*	*2.053*
C.citratus	*66.60±0.68*	*0.445*	*67.70±1.28*	*1.013*
P.guineense	*68.95±0.79*	*2.35*	*65.50±1.17*	*1.130*

Control value= 66.95±0.40 IU/L

*= Mean+S.D values for 4 rats.

Table 4: Effects Of Extracts On Sakp

	DAY 7		DAY 14	
Aqueous Extracts	Mean+S.D*	P value	Mean+S.D*	P value
P.guajava	*23.23±069*	*0.562*	*21.44±0.77*	*0.354*
E.aromatica	*23.11±0.41*	*0.162*	*23.72±0.68*	*0.422*
D.benthmianus	*25.21±0.41*	*1.679*	*24.81±0.36*	*1.471*
Z.officinales	*22.46±0.37*	*0.176*	*23.56±1.17*	*0.639*
P.guineense	*20.35±0.88*	*0.88*	*19.35±0.99*	*1.613*
C.citratus	*25.66±0.82*	*0.758*	*21.06±0.47*	*0.584*

Control value= 22.14±1.53 IU/L

*= Mean+S.D values for 4 rats.

Table 5: Effects Of Extracts On Sacp

	DAY 7		DAY 14	
Aqueous Extracts	Mean+S.D*	P value	Mean+S.D*	P value
P.guajava	*105.45±0.81*	*0.428*	*102.23±1.22*	*0.324*
E.aromatica	*100.57±0.50*	*0.740*	*99.34±1.40*	*1.025*
D.benthmianus	*112.31±1.07*	*2.015*	*110.52±1.42*	*1.551*
Z.officinales	*99.81±0.54*	*0.919*	*98.20±0.66*	*1.298*
P.guineense	*105.45±1.04*	0.437	*99.31±1.46*	*0.972*
C.citratus	*105.66±1.33*	*0.459*	*106.69±1.00*	*0.713*

Control value= 103.64±3.57 IU/L *Mean±S.D values for 4 rats.

Table 6: Effects Of Extracts On Sld

	DAY 7		DAY 14	
Aqueous Extracts	**Mean+SLD***	**P value**	**Mean+SLD***	**P value**
P.guajava	*17.21±5.21*	*0.129*	*17.37±19.34*	*0.078*
E.aromatica	*17.35±4.60*	*0.170*	*17.22±11.60*	*0.107*
D.benthmianus	*17.70±2.44*	*0.814*	*1792±28.27*	*1.151*
Z.officinales	*17.78±2.67*	*0.919*	*16.93±11.37*	*0.710*
C.citratus	*17.19±2.11*	*0.170*	*17.39±4.12*	*0.259*
P.guineense	*16.41±1.27*	*1.779*	*17.22.±11.66*	*0.104*

Control value= 17.27± 3.99 IU/L *= Mean+S.D.

TABLE 7: Effects Of Extracts On Direct And Total Bilirubin.

	DAY 7		DAY 14	
Aqueous Extracts	Mean+S.D*	P value	Mean+S.D*	P value
P.guajava	0.35 ± 0.01^a	*0.278*	0.34 ± 0.019^a	*0.546*
	0.81 ± 0.02^b	*0.614*	0.79 ± 0.03^b	*1.00*
E.aromatica	0.34 ± 0.02^a	*0.480*	0.33 ± 0.019^a	*0.541*
	0.81 ± 0.02^b	*0.514*	0.80 ± 0.02^b	*0.804*
D.benthmianus	0.39 ± 0.02^a	*0.721*	0.37 ± 0.01^a	*0.274*
	0.84 ± 0.02^b	*0.306*	0.82 ± 0.01^b	*0.387*
Z.officinales	0.37 ± 0.01^a	*0.274*	0.38 ± 0.01^a	*0.548*
	0.77 ± 0.02^b	*1.837*	0.81 ± 0.04^b	*0.387*
C.citratus	0.34 ± 0.02^a	*0.480*	0.34 ± 0.02^a	*0.480*
	0.82 ± 0.01^b	*0.387*	0.80 ± 0.01^b	*1.162*
P.guineense	0.32 ± 0.02^a	*0.959*	0.29 ± 0.01^a	*1.904*
	0.77 ± 0.02^b	*1.845*	0.85 ± 0.03^b	*0.479*

Control value= 0.36 ± 0.03^{1} mg/dL^{a} 0.83 ± 0.02^{2} mg/dL^{b} * Mean+S.D values for 4 rats. a and b - Direct and Total respectively The above results did not reveal any appreciable difference between neat animals and those administered the aqueous extracts. Two obvious issues are apparent - the dosage and the health status of the animals. At the dosage used in these experiments, there is no apparently deleterious effect. The organ markers in the serum, points to the liver, kidney, muscle, bone and erythrocytes of the animals. The implications of diseases have to be verified for correct and complete insight on the effects of medicinal plants.

DISCUSSION AND CONCLUSION

Our investigation was set to provide more information on the rational for the uses of medicinal plants by the locals . We screened six (6) apparently medicinal plants parts: *Psidium_guajava,* leaves, *Eugenia aromatica* cloves, *Distemonanthus benthmianus* stembark, *Zingiber officiale* fruit *Piper guineense* berry and *Cymbopogon citratus* leaves. These were chosen not because they elicit the same medicinal properties but that they are commonly used as first choice by the locals, (Akindewo, 1999),because of their assessibility. The risk and impact on the populace could be subtly devastating if they harbour potentially hazardous phytoconstituents hence, we screened them for alkaloids, tannins, flavonoids, glycosides, saponins and reducing sugars. Our findings, revealed that all the extract lacked cycinogenic glycosides, hence absence of toxic effects. However they contain cardiac glycosides. These probably are very low in concentration when compared with other phytoconstituents, otherwise they are potentially poisonous xenobiotics. (Trace and Evans, 1985). Cardiac glycosides often irritates the gastric mucosa and leads to nausea and justifies their uses as emetics and expectorants (Bep,1960). However, the plants screened were not known to be used for these purposes, suggesting that the concentration of the glycosides could be low indeed. Apart from this possibility, whereas there are at least two (2) tests for other phytoconstituents, cardiac and cyanogenic glycosides seems to have just one confirmatory tests. We suggest that pharmaceutical chemists should help look into the possibility of detecting glycosides other than the tests used currently. This will help to track the real cause of debilitating effects of medicinal plants, where the tests used in our study reveal nothing. All the aqueous extracts confirm the presence of alkaloids. This is not surprising, alkaloids are by far the largest class of nitrogenous compounds in plants. The precursors are amino acids although their biosynthesis is complex {Tedder,et al.1972}. All the extracts contains apart from alkaloids; tannins, flavonoids saponins and reducing sugars.

Indeed, sometimes glycosides and saponins yield glucose and other reducing sugars on hydrolysis. This explains why they contain reducing sugars. Tannins are used for their astringent properties in the treatment of scalds and burns (Tedder,etal.1972.). Apparently the concentrations of these phytoconstituient are high and probably explains why they are used by the locals.

Our result gave no reason to suggest bioaccumulation, with its potential side effects, despite the daily administration of extracts to rats. This might be because of the high turnover rate in systemic circulation. It is probable that the extracts are easily digested and absorbed by the cells or they are unstable and are broken down and excreted. Either of these is probable. The systemic studies on rats over 14 days, Tables 2 to 7, did not reveal any noticeable deleterious effect on the organs of the liver, heart, kidney, muscles and the erythrocytes. The roles of flavonoids- as anti-oxidants, enzyme inhibitors and antibacterials are benefits that should be exploited. These are conferred on the plants that posses them. Although, they are also precursors of toxic substances, our results did not subject the presence of these in the extracts studies.

It is necessary to note that this studies was on acute administration of these aqueous extracts. Deleterious effects may result from chronic administration or abuse of administration by the locals, hence caution should be exercised with the extracts. The apparent high clearance rate (turnover) by the xenobiotics metabolising organs may not be reliable when the organ functions are compromised during infections or other diseases. A pathologic

organ might not be able to meet the requirement for metabolism of these phytoconstituent with the result of toxicities or any other deleterious consequence. These however are areas that could be looked into. How the organs respond to phytoconstituents, when they are diseased could also be looked into.

ACKNOWLEDGEMENT

We wish to acknowledge the assistance of Mr. Ojo of the Laboratory Animal Centre, and Mr. Akinrodoye of the Biochemistry Department of the College of Medicine of the University of Lagos, during the analysis of serum enzymes and bilirubin.

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