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(RESEARCH ARTICLE)

Phytochemical compositions and biochemical effect of *Phyllanthus amarus* in albino rat

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Abstract

Phyllanthus amarus is a commonly known weed used for the traditional treatment of several aliment. In the present study, phytochemical screening and toxicological profile of methanol and aqueous extracts of *P. amarus* were investigated. Qualitative and quantitative phytochemical analysis were carried out using standard procedures. Sub-acute toxicity was carried out by oral administration of the aqueous extract at 300 and 600ml/kg for 14days. Biochemical parameters including aspartate amino tranferases (AST), alanine amino transfereas, (ALT), alkaline phosphates (ALP) albumin, total protein and albumin were investigated. The results obtained showed the presence of alkaloids, phenols, tannin, flavonoids, saponins, glycosides and steroids in both extracts. Quantitatively, alkaloids (0.56±0.01 and 0.56±0.00 mg/g) were the most abundant phytochemical while phenol (0.09±0.01 and 0.07±0.01 mg/g) was the least abundant in both extracts. The concentrations of total proteins were significantly ($p<0.05$) lowered in rats dosed with 600 mg/kg bw of the extract when compared with the control rats. However, serum activities of ALT, AST, ALP and albumins were not significantly ($p>0.05$) altered when compared with the control values. Methanol and aqueous extracts of *P. amarus* contains important phytochemicals with therapeutic reputations. The extract was also found not to have adverse/toxic effect on liver integrity at doses of up-to 600 mg/kg bw in rats.

Keywords: Biochemical; Acute and sub-acute toxicity; *P. amarus*; Phytochemicals

1. Introduction

Natural products are important sources of bioactive metabolite with varied structural and therapeutic effect [1]. Decade of research has witnessed surfeit of research on therapeutic and medicinal properties of natural product owing to unsatisfactory and toxicity of synthetic drugs [1-3].

Phyllanthus amarus is a flowering plant of family Phyllanthaceae. It is highly distributed in most tropical and sub-tropical countries. It is commonly known as *yin-olobe, geron tsuntsaye, susuma suyengi* and *Ngwu ite kwowa nasu by* the Yorubas (South-Western), Hausas (Northern part), Nupes and the Igbos of the South-East of Nigeria respectively [4]. Previous phytochemical study of *P. amarus* confirmed the presence of bioactive metabolites including phyllantine, hypophyllantine alkaloids and flavonoids like quercetin, with diverse therapeutic values [5]. It is

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commonly used in folk medicine for treatment of several diseases including hepatitis, malaria, fever, diabetes, stomach upset, cold anaemia, eczema, jaundice and as a remedy for blood disorders [6].

Extract of *P. amarus* exhibited antiplasmodial [7], cytotoxic activities [8], antidiarrheal [9], antioxidants [10] and hypolipidemic [5], effect. It is however, recommended that in addition to therapeutic values of plants, safety profile should be a major overriding criteria for the selection and uses of plant for health care need. It is in the view of this, that the present study was design to evaluate the phytochemical constituents as well as biochemical effect on liver integrity in rats.

2. Material and methods

2.1. Collection and identification of plant materials

Fresh leaves of *Phyllanthus amarus* were collected around the Federal University of Technology Minna Bosso campus on 24th March, 2017 and authenticated by a Botanist at Biological Science Department, Federal University of Technology Minna. The leaves were washed under running water, air dried and pulverized to obtain powdered form that was used for study. Chemicals and Reagents used were of analytical grade

2.2. Experimental animal

Total of twenty-five (25) Wister albino rats average weight 150.56±5.70g were obtained from Animal house of School of life sciences, Federal University of Technology Minna. The rats were kept in the Laboratory under favuorable atmospheric conditions (37 °C) and acclimatized for two weeks before being used for the experiment. They were allowed accessed to commercial grower feed (Vital feed) and water ad-libitium.

The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

2.3. Preparation of aqueous and methanol extracts

Air-dried sample of *P. amarus* (150 g each) was extracted separately with 500 ml of methanol using Soxhlet apparatus for 3 hours. Methanol extracts obtained was decanted and evaporated in rotatory evaporator at 65 ^{o}C respectively under a reduced pressure. Distilled water was used for the aqueous extraction using cold maceration. The aqueous extract was then filtered and evaporated using water bath. The concentrated extract obtained was preserved in sterile glass container and kept in refrigerator until the required time for use.

2.4. Qualitative and quantitative phytochemical analysis

Phytochemical analysis of extracts were carried out for the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenols and phlobatannins in methanol and aqueous extracts of *P. amarus* according to methods of [11]. Quantitative analysis was conducted for flavonoid, alkaloids, total phenol, tannin and saponins using standard procedures [12]

2.5. Acute toxicity study

Acute oral toxicity study was performed according to OECD –423 guidelines [13]. A total of fifteen (15) overnight fasted albino rats were randomly grouped in to three (5 each). Each extract was orally administered once at dose of 2500 mg/kg body weight. Control group was given 0.2 ml normal saline. The rats were observed every 30 minutes for 4 hours for detailed behavioral, neurological profile, delayed toxicity or mortality every day for 14 days.

2.6. Sub-chronic Toxicity

A total of twenty (20) rats were randomly divided into four groups of 5 rats each as follows: Each group was separately housed.

- Group A — Control groups that received no treatment.
- Group B — were administered 150 mg/kg aqueous extract of *P. amarus*
- Group C — were administered 300 mg/kg aqueous extract of *P. amarus*
- Group D — were administered 600 mg/kg aqueous extract of *P. amarus*

All treatments were administered orally once daily for 14 days, after the 14th days of the treatment with extract, the rat were then sacrificed.

2.7. Collection of blood sample and analysis of biochemical parameters

Collection of sample for biochemical analyses was as described previously [14]. The animals were anaesthetized with ether and blood was collected through cardiac puncture into a clean, dry EDTA- free centrifuge tubes. The blood samples were allowed to stand for 10 minutes at room temperature and then centrifuged at 1000 rpm (503 x g) for 15 minutes to get the serum. Serum biochemical parameters including aspartate aminotransferase (AST), Alanine Aminotransferase (ALT), alkaline phosphatase, total proteins and albumin concentration were assay using standard procedures [15-17].

3. Results

3.1. Phytochemicals

The aqueous and methanol leaf extract of *P. amarus* contains alkaloids, phenols, tannis, flavonoids saponins, glycosides and steroids. Terpenoids was absent in methanol while phlobatannins was absent in both extracts (Table 1). Quantitatively, alkaloids were the most abundant phytochemicals while phenol was the least abundant in both extracts. The aqueous and methanol leaf contains 0.33±0.01 and 0.28±0.01 mg/g of flavonoids, 0.56±0.01 and0.56±0.00 of Alkaloid; 0.41±0.04 and 0.34±0.02 of Saponin; 0.09±0.01 and 0.07±0.01 of phenol; 0.34±0.05 and 0.26±0.03 of tannins respectively (Table 2).

Table 1 Phytochemical composition of *Phyllanthus amarus*

Phytochemcial	Aqueous	Methanol
Alkaloid	+	+
Glcoside	+	+
Steroids	+	+
Tannins	+	+
Saponins	+	+
Phlobatannins	-	-
Terpenoids	+	-
Phenol	+	+
Flavonoids	+	+

Key + = Detected - = Not Detected

Table 2 Quantitative phytochemical composition of methanol and aqueous extract of *P. amarus*

Parameter	Methanol (mg/g)	Aqueous(mg/g)
Flavonoid	0.33±0.01 ^{b}	0.28±0.01^{b}
Alkaloid	0.56±0.01 ^{d}	0.56±0.00^{d}
Saponin	0.41±0.04 ^{c}	0.34±0.02^{c}
Phenol	0.09±0.01^{a}	0.07±0.01^{a}
Tannin	0.34±0.05 ^{b}	0.26±0.03b

Mean ± Standard Error of triplicate determination. Values with different superscripts along a column are significantly different (P<0.05)

3.2. Acute toxicity test (limit test)

For both aqueous and methanol extract, the behavioral profile (alertness, restleesness, irritability, fearfulness), neurological such as (reactivity, touch response and pain response) and autonomic profile (defecation and urination) were all normal during the side cage observation and beyond. Although they were less active for the first six hours but their activities became normal afterward. No mortality was recorded during the fourteen days of observation (Table 3).

Table 3 Acute toxicity test of *P. amarus* (Limit Test)

Group dose	Extract activity			
	Behavioral profile	**Neurological profile**	**Autonomical profile**	**Mortality**
A	Normal	Normal	Normal	Nil
B	Normal	Normal	Normal	Nil
C	Normal	Normal	Normal	Nil

Group A: rats that received 2500 mg/kg bw of methanol extracts of *P. amarus*
Group B: rats that received 2500 mg/kg bw of Aqueous extracts of *P. amaru*
Group C: rats that received no extract it serve as control

3.3. Biochemical parameters

The concentrations of total proteins were significantly ($p<0.05$) lowered in rats dosed with 600 mg/kg bw of the extract when compared with the control rats. However, serum concentrations of ALT, AST, ALP, total proteins and albumins were not significantly ($p>0.05$) altered when compared with the control values (Table 4).

Table 4 Effects of aqueous extract of *P. amarus* on biochemical parameters in albino rat

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (mg/dL)	Albumin (mg/dL)
150 mg/kg *P. amarus*	48.84±4.34^a	70.89±4.32^a	231.78±7.64^a	70.43±3.84^b	4.76±0.29^a
300 mg/kg *P. amarus*	56.34±4.24^a	72.22±6.34^a	220.56±13.01^a	72.97±4.99^b	4.98±0.06^a
600 mg/kg *P. amarus*	49.90±5.77^a	73.01±8.02^a	233.24±11.87^a	56.31±7.94^a	4.86±0.53^a
CONTROL	49.97±5.05^a	70.01±6.24^a	236.03±15.67^a	72.48±1.11^b	4.76±0.36^a

Values are mean ± SEM of 5 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

4. Discussion

The present study revealed the presence of various important medicinal phytochemicals in aqueous and methanol leaves extract leaf extract of *P. amarus.* The presence of flavonoid, tannin, saponin, steroid has been previously reported for methanol extract [6], and aqueous extract [11] of *P. amarus.* The phytochemicals identified in this study particularly, flavonoids, phenols and alkaloids (which is the most abundant phytochemicals in both extract (0.56±0.01 and 0.56±0.00 mg/g) are well known for diverse pharmacological activities [18]. Therefore, the present of these phytochemicals in aqueous and methanol leaf extract of *P. amarus* is an indication that this plant if properly screened could yield a drug of pharmaceutical significance. The higher amount of phytochemicals in methanol extract compared to that of aqueous extract indicated the bioactive agent in *P. amarus* are more soluble in polar organic solvent, this may confer higher biological activities to the methanol extract. However, the absence of terpenoids in methanol extract and the absence of Phlobatannins in both samples agree with early studies which also found that not all phytochemicals are present in all plant and those that present differs according to the solvent use in the extraction process [19]

In attempt to evaluate the safety or toxicity of plant extract, evaluation of biomarkers of organs integrity become relevant as it provides adequate diagnostic, prognostic information as well as pathological condition of animals exposed to the text substance [20]. In the present study, there was no test substance related mortality observed at limit dose of 2500 mg/kg. Therefore, *P. amarus* could be generally regarded as safe (GRAS). This finding is in agreement with Clarke and Clarke [21], who reported that any compound or drug with oral LD50 estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe.

Alkaline phosphatases are often used to assess the integrity of plasma membrane and endoplasmic reticulum [22]. The observed non-significant difference from the control values in the activities of alkaline phosphatase (ALP) after 2 weeks of administrations of aqueous extract of *P. amarus* suggested that the integrity and functionality of endoplasmic reticulum and plasma membrane has not been comprised [23]. It also indicates that the extract did not inhibit or activate the activities of the enzyme molecule in situ [24]. AST and ALT are biomarkers of hepatic integrity and to a certain level can be used to assess the extent of hepatocellular damage, the ALT activities however, give more valuable information relevant to the integrity of the hepatocyte than AST [15]. The insignificant ($p>0.05$) difference in the activities of AST and ALT in rats following administration of aqueous extract of *P. amarus* suggests that there were no leakages of these

enzymes from liver into the serum. This indicated that hepatic metabolism of the *P. amarus* extract and tissue turnover, is not adversely affected [25].

The level of total protein plays important roles in determining the synthetic and excretory roles of the kidney and liver [15]. The results obtained in this study indicated that albumins and total proteins were not adversely effected by the extract administration at doses of 150 and 300 mg/kg bw. The observed decrease in the total proteins content in rats dosed with aqueous extract of *P. amarus* (600 mg/kg) for 2weeks suggests that the extract might have interfered with the equilibrium in the rate of synthesis or destruction of total protein from the system of the animals [26]. Such decrease could, however, lead to hydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals [21].

5. Conclusion

The findings of the present study indicate that methanol and aqueous extracts of *P. amarus* contains important phytochemicals of therapeutic application. The extract was also found not to have adverse/toxic effect on liver integrity at doses of up-to 600 mg/kg bw as revealed by biomarker enzyme, thus its safe for consumption.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of ethical approval

The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

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