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Full Length Research Paper

Assessment of the hepatic effects, phytochemical and proximate compositions of *Phyllanthus amarus*

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Aqueous extract of pulverized whole *Phyllanthus amarus* was assessed for hepatic effects in albino rats. Its phytochemical, proximate and mineral constituents were also evaluated. The results showed that the extract significantly reduced the plasma activities of alanine and aspartate transaminases and total bilirubin concentration ($P < 0.01$), with a non- significant increase in the plasma concentration of total protein ($P > 0.01$). The extract contained 24.05% saponins, 17.50% tannins and 5.47% oxalates as well as 11.05% moisture, 6.80% ash, 6.03% fat, 6.10% protein, 24.50% fibre and 45.52% carbohydrate. The mineral content of the defatted pulverized *P. amarus* was found to be potassium (150.30), sodium (228.20), calcium (1.60), magnesium (2.40), iron (1.65), and phosphorus (1.00) mg per 100 g dry weight. The reduction of plasma activity of transaminases and concentration of total bilirubin, with a concomitant increase in total protein concentration suggest that the plant has a hepatic cell protection function and enhancement potential. The high saponin and tannin, potassium and sodium, and carbohydrate and fibre contents of the plant explain its use in folk medicine for the treatment of liver problems, oedema and use as tonic, respectively.

Key words: Phyllanthus amarus, chemical composition, hepatic effects, albino rats.

INTRODUCTION

Phyllanthus amarus is a plant of the family Euphorbiaceae. It was first identified in central and southern India in 18th century but is now found in many countries including Philippine, Cuba, Nigeria among others. It is commonly called 'carry me seed', 'stone-breaker', 'windbreaker', 'gulf leaf flower' or 'gala of wind'. *P. amarus* is an erect annual herb of not more than one and half feet tall and has small leaves and yellow flowers. In folk medicine, *P. amarus* has allegedly been used to treat jaundice, diabetes, gonorrhea, irregular menstruation, tachycardia, dysentery, spasmodic cough, itchiness, arthritis, otitis, swelling, skin ulcer and weakness of male organ (Calixto et al., 1998). In India, its aqueous preparations are used as a good tonic taken safely at home along with native food (Thyagarajan et al., 1988). Infusion of leaves, stem and root of *P. amarus* are used in Brazilian folk medicine for treating kidney problems, intestinal infection and liver problems (Calixito et al., 1998). It has also been alleged that *P. amarus* aqueous extract is used in Nigerian homes to eliminate waste from the body, restore the activity of the liver and build up blood and innate defense system. It is also used for inducing labour and treatment of oedema, feverish pain, sore throat, female sterility, oliguria and vaginitis (Calixto et al., 1998). Furthermore, extracts from *P. amarus* such as tannins and phenol have been associated with some medical importance. It contains tannins with antimicrobial activity. There are also hydrolysable tannins, which have been found to inhibit DNA polymerase and reverse transcriptase in HIV infection, and angiotensin-converting enzymes involved in diabetes complication (Bensky and Gamble, 1993).

There is at present little information available on the adverse effects, phytochemical, mineral and proximate compositions of aqueous extract of *P. amarus*. The Importance of such information cannot be over-emphasized since the plant's extract is taken as a tonic in many homes and many local medicinal preparations are not properly evaluated before use. In this communication, the phytochemical, proximate and mineral compositions of the plant are reported as well as the hepatic effects of

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its aqueous extract on albino rats.

MATERIALS AND METHODS

Collection and preparation of plant sample for analysis

The samples of whole *P. amarus* used for this work were collected from their natural habitat in and around Federal University of Technology, Owerri compound. The plant leaves were identified by Dr. S. E. Okeke, a plant Taxonomist of the Department of Plant Sciences and Biotechnology, Imo State University Owerri, Nigeria. They were thoroughly washed and rinsed in distilled water, and sun-dried. The dried plant samples were ground to fine powder with a domestic electric grinder, packaged in glass jars and stored in a refrigerator at 4°C until required for use.

10 g of the *P. amarus* fine powder were dissolved in distilled water and the volume made up to 100 ml. The solution was boiled for 30 min, allowed to cool and then filtered. The residue was washed with distilled water through the filter paper into the filtrate until the volume is 100 ml. This formed the 10% aqueous extract.

Experimental animals and biochemical analysis

Ten male wistar albino rats of mean weight 98.0 ± 5.6 g, obtained locally from Nsukka, Enugu State, Nigeria were housed in metal cages. The rats were weighed and divided into two groups (test and control) of five rats each, with equal average group body weights (98.0 g). They were fed ad libitum water and growers' mash (Guinea Feed Nigeria Ltd.) for about a week to equilibrate them to laboratory conditions. After acclimatization, the test animals were subjected to oral treatment with the aqueous extract (4 ml/rat/day) while the control animal were given normal saline (4 ml/rat/day), using a feeding tube attached to a 5 ml syringe for a period of 28 days. Meanwhile, all the animals were still placed on water and grower's mash ad libitum throughout the period. At the end of the experimental period, the animals were sacrificed by exposure to chloroform in an enclosed container. Incisions were quickly made into the sacrificed animal's cervical region with the aid of a sterile blade and blood samples collected from the heart and dispensed into heparin-containing bottles. The plasma obtained from the blood samples were used for the liver function tests. Alanine transaminase (ALT) and aspartate transaminase (AST) activities as well as total bilirubin, conjugated bilirubin and total protein concentrations were determined as described by Balistreri and Shaw (1987). The unconjugated bilirubin concentration was calculated as the difference between total and conjugated bilirubin concentrations.

Phytochemical analysis

Quantitative determination of saponins, tannins, alkaloids and cyanogenic glycosides were carried out according to the methods described by Harborne (1973) and Trease and Evans (1983). Oxalate contents were determined by the spectrophotometric methods of Hang and Lantzsch (1983). Determinations were done in triplicates.

Proximate composition

The ash and moisture contents were determined as described by AOAC (1990). Crude fat was extracted by the Soxhlet method with petroleum ether (40 - 60°C) for 8 h. The total nitrogen was determined using the microjeldahl method and converted to crude protein content by multiplying with 6.25. The carbohydrate content was determined by the percentage difference of the various other proximate compositions summed together. Determinations were done in triplicates and results expressed as averages of percent values on dry weight basis.

Mineral composition

The sample was incinerated into ash, dissolved in 1 ml of 2 M HCl and diluted to 100 ml with deionized water. The resulting extract was used for the determination of sodium, potassium, iron, calcium and magnesium by the use of an atomic absorption spectrophotometer (Perkin Elmner, USA). Phosphorus was determined as phosphate by the vanadomolybdate colorimetric method (Pearson, 1976).

Statistical analysis

Data obtained were analyzed by the use of Student's t-distribution test and values for $P < 0.01$ were considered statistically significant.

RESULTS

The plasma concentrations of the liver function parameters are given in Table 1. The values obtained showed that the ALT and AST activities decreased significantly in the blood of the test animals (26.13 ± 5.01 and 33.75 ± 5.30 U/l, respectively) when compared to those of the control group (48.28 ± 5.68 and 56.43 ± 6.12 U/l, respectively) ($P < 0.01$). Similarly, the concentrations of total and unconjugated bilirubin decreased significantly ($P < 0.01$), while concentration of total protein increased non-significantly in the test group ($P > 0.01$).

Table 2 presents the phytochemical constituents of P. amarus. It showed that the plant contained high levels of saponins and tannins at 24.05 and 17.50%, respectively, but with low content of cyanogenic glycosides (1.46%). The proximate composition is given in Table 3. The plant contained high percentage level of fibre (24.50%) and carbohydrate (45.52%), with approximately content of fat (6.03%, protein (6.10%) and ash (6.80%). Some of the mineral constituents of the plant are listed in Table 4. The potassium and sodium contents were high at 150.30 and 228.20 mg per 100 g dry weight, respectively, while magnesium, calcium, iron and phosphorus were all low at 2.40, 1.60, 1.65 and 1.00 mg per 100 g dry weight, respectively.

DISCUSSION

In recent years, substantial interest has been placed on the chemical and pharmacological properties of the plants of the genus Phyllanthus that have long history in folk medicine, especially in traditional Chinese medicine, where they have been used to treat kidney and urinary bladder disturbances, intestinal infections, diabetes and hepatitis B infection (Calixto et al., 1998). The results of

Table 1. Plasma levels of liver function parameters in the albino rats fed aqueous extract of *Phyllanthus amarus*.

Parameter	Test*(n = 5)	Control*(n = 5)	t - value	Remark
ALT (U/l)	26.13 ± 5.01	48.28 ± 5.68	5.85	P < 0.01
AST (U/l)	33.75 ± 5.30	56.43 ± 6.12	5.60	P < 0.01
Total Bilirubin (µmol/l)	7.01 ± 0.14	9.23 ± 0.06	16.02	P < 0.01
Conjugated Bilirubin (µmol/l)	1.19 ± 0.01	0.96 ± 0.01	32.53	P < 0.01
Unconjugated bilirubin (µmol/l)	5.83 ± 0.08	8.28 ± 0.04	54.81	P < 0.01
Total protein (g/l)	54.20 ± 0.80	53.30 ± 0.91	1.49	P > 0.01

*Values are mean ± standard deviation.

Table 2. Phytochemical constituents of *Phyllanthus amarus* extract.

Phytochemical	Composition (%)*
Saponins	24.05 ± 0.21
Tannins	17.50 ± 0.18
Oxalates	5.47 ± 0.08
Alkaloids	2.56 ± 0.02
Cyanogenic glycosides	1.46± 0.06

*Mean of triplicate determinations ± standard deviation.

Table 3. Proximate composition of *Phyllanthus amarus* extract.

Component	Composition (%)*
Moisture	11.05 ± 0.08
Ash	6.80 ± 0.03
Fat	6.03 ± 0.04
Protein	6.10 ± 0.02
Fibre	24.50 ± 2.15
Carbohydrate	45.52 ± 4.81

*Mean of triplicate determinations ± standard deviation.

Table 4. Mineral composition of *Phyllanthus amarus* extract.

Element	Concentration (mg/100 g dry weight)*
Potassium	150.30 ± 2.54
Sodium	228.20 ± 4.12
Calcium	1.60 ± 0.02
Magnesium	2.40 ± 0.03
Iron	1.65 ± 0.02
Phosphorus	1.00 ± 0.01

*Mean of triplicate determinations ± standard deviation.

the present study on *P. amarus* show that it has the ability to enhance the bilirubin conjugating and protein synthesizing functions of the liver as elucidated by the decrease in blood total and unconjugated bilirubin as well as an increase in blood protein concentration of the test animals. In the same vein, it has the potential of preventing hepatic cell destruction, which will usually be marked by an increase in blood transaminases. These findings may explain better the earlier observed protective action of the plant's extracts against chemically induced and microbial injuries of liver cells (Jayaram and Thyagarajan, 1996; Rajeshkumar and Kuttan, 2000).

Phytochemicals occur in various parts of plants. Their functions are diverse which include provision of strength to plants, attraction of insects for pollination and feeding, defense against predators, provision of colour, while some are simply waste products (Ibegbulem et al., 2003). These secondary plant metabolites exhibit varied biochemical and pharmacological actions in animals when ingested (Trease and Evans, 1983). Saponins are known to have hypocholesterolemic activities (Price et al., 1987). The high content of saponin in the plant may also aid in lessening the burden on the liver as regards cholesterol metabolism, thereby allowing it, more room to recuperate when challenged. Oxalates from plant sources have been known to cause irreversible oxalate nephrosis when injested in large doses. The presence of oxalates in the extract may sound a note of caution in the ingestion of the plant extract. However, the low concentration observed may not pose danger. Meanwhile, the presence of minerals such as calcium in the plant can ensure adequate removal of these anti-nutritional factors by formation of complexes. Complex formation between calcium and oxalate makes more calcium minerals unavailable; however it also ensures excretion of oxalates. Furthermore, steaming or boiling reduces oxalate content of plant extracts to very minimal concentrations (Piorreck et al., 1984).

Although, folk history does not have a record of *P. amarus* being used as a vegetable, the extract contains a high percentage composition of carbohydrates and crude fibre. This may be the reason why the plant's extract is allegedly used as tonic (Thyagarajan et al., 1988), because of its high and readily available carbohydrate content. The appreciable concentration of minerals such as sodium, potassium, calcium and magnesium observed in the plant is interesting. These minerals function, among other areas, in the maintenance of osmotic pressure and water distribution in the various body fluid compartments. This explains the traditional use of the plant extract in the treatment of oedema, kidney problems and oliguria (Calixto et al., 1998).

In conclusion, the study has revealed that the extract of *P. amarus* has hepatic cell function enhancement ability, which may explain its use traditionally in the treatment of liver problems. It also has high content of carbohydrate, potassium and sodium, which is related to the plant's alleged use as tonic and in the treatment of oedema and kidney disorders.

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