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Research Article

PHYTOCHEMICAL, ANTIMICROBIAL AND TOXICITY STUDIES OF *PHYLLANTHUS AMARUS* WHOLE PLANT EXTRACT

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Abstract: This study investigated the phytochemical composition, antimicrobial activities and toxicological properties of methanolic whole plant extract of *Phyllanthus amarus*. Phytochemical analysis revealed the presence of saponins, tannins, flavonoids, terpenoids and cardiac glycosides. Antimicrobial sensitivity test using fourteen human pathogenic microorganisms showed that *Phyllanthus amarus* has bactericidal effect on four pathogens namely *Proteus vulgaris*, *Micrococcus luteus*, *Bacillus polymyxa* and *Escherichia coli*. The extract also demonstrated significant inhibitory effect on *Corynebacterium pyrogenes*, *Bacillus subtilis*, *Clostridium sporogenes*, *Klebsiella pneumonia* and *Staphylococcus aureus* but the microbes were able to recover after a few hours. Administration of *Phyllanthus amarus* extract to rats for 14 days caused growth depression with no significant change ($P< 0.05$) in serum concentrations of urea, creatinine, bilirubin and aminotransferases (ALT and AST) compared to untreated group. Measurement of organ: body weight index did not show any indication of kidney or liver enlargement. These result shows that *Phyllanthus amarus* leaf contains active phytochemicals which are inhibitory to bacteria and might be useful in the treatment of microbial infections in man. It also indicates that the extract might not be toxic to the kidney and the liver.

Keywords: *Phyllanthus amarus*, Antimicrobial, Phytochemicals, Kidney Function, Liver Function

INTRODUCTION

The prevalence of multi-drug-resistant pathogens has continue to threaten the clinical efficacy of many existing antibiotic drugs leading to extensive investigation of medicinal plants for potential antimicrobial activity in recent years [1,2]. Some plants have been identified to contain medicinal constituents which have potentially significant therapeutic applications against human pathogens including bacteria, fungi and viruses [3].

Phyllanthus amarus (Eyin olobe in Yoruba) which belong to the family Euphorbiaceae is a small, erect, annual herb having large number of phytochemicals that are attributed to its leaves, stem and roots. The herb is a distinguished plant worldwide which has been used over the years because of its rich medicinal component [4]. *Phyllanthus amarus* is traditionally used among different ethnic groups in Nigeria for the treatment of jaundice, diarrhea, dysentery, diabetes, fevers, uro-genital diseases, ulcers, sores, boils and wounds [5, 6]. Juice from the roots and leaves are taken internally to stimulate the kidney. Previous findings have revealed that extracts from different parts of *Phyllanthus amarus* demonstrated anti-oxidant, anti-inflammatory, hypocholesterolemic anti-carcinogenic and anti-HIV potential [7,8,9,10].

Despite the fact that a number of laboratory and clinical studies have been conducted on the therapeutic efficacy of this plant, there is no reported toxicity investigation on it especially *Phyllanthus amarus* growing in Nigeria. We intend to determine the major phytochemicals present in this plant as well as evaluating its antimicrobial efficacy. Bio-safety of this plant species growing in Nigeria will also be ascertained since plant composition of the same species may vary in different geographical location.

MATERIALS AND METHOD

Plant collection and preparation of plant extract:

Phyllanthus amarus whole plant was collected in Oke-Baale area of Osogbo, Osun State Nigeria. The plants were air-dried at room temperature to a constant weight for six weeks and then pulverized using an electric blender. The powder was soaked in 80% methanol (1:6 v/v) for 14 day after which it was filtered using Whattman filter paper. Crude extract was obtained by evaporating the solvent in a water bath at 40°C.

Phytochemical screening:

Phytochemical analyses was carried out on the powdered sample and the extracts for the presence of tannins, phlobatannins, flavonoids, steroids, terpenoids, saponins and cardiac glycosides using earlier described standard procedures [11,12,13].

Preparation of pure isolates:

Pure isolates of 14 human pathogenic microorganisms were cultured in test tubes containing

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sterilized nutrient agar for 18 hours in an incubator. The isolates include *Bacillus anthracis, Escherichia coli, Bacillus subtilis, Clostridium sporogenes, Pseudomonas aenogenes, Klebsiella pneumonae, Streptococcus faecelis, Staphylococcus aureus, Micrococcus luteus, Bacillus cereus, Corynebacterium pyrogenes, Bacillus polymyxa, Bacillus stearothermophillus and Proteus vulgaris*. Mueller-Hinton agar was prepared into 14Mc Carteny bottles and sterilized using an autoclave at 121°C for 15 minutes.

Antimicrobial Investigation:

Antimicrobial sensitivity testing was carried out on the extracts as well as Ciprofloxacin, a standard antibiotic using the broth dilution assay procedure [14] and their zones of inhibition measured. A cloudy solution indicated the presence of the pathogens in each solution. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the extract was determined for sensitive pathogens following standard method [15].

Experimental animals:

Eighteen Wistar strain albino rats (average weight 120g) were used for these studies. They were obtained at the Department of Physiology, University of Ibadan and housed in well ventilated cages in the Central Animal House, Osun State University, Osogbo, Nigeria in an optimum laboratory condition. The rats were sorted into three groups (A, B and C) of 6 rats each. Rats in group A served as the control and were administered distilled water daily. Group B and C rats received 200mg/kg bw and 500mg/kg bw respectively of *Phyllanthus amarus* extract. The extract was administered orally daily for 14 days.

Serum collection and toxicological analysis:

Rats were sacrificed at the end of 14 days by cervical dislocation. Blood were collected into sterilized plain bottles and left for 5 minutes to clot. It was then centrifuged at 3000rpm for 20 minutes to obtain a clear supernatant (serum). The animals were quickly dissected to remove the kidneys and liver which were weighed to determine organ: body weight index. Urea concentration in the serum was determined based on fearon reaction [16]. Creatinine concentration was determined based on Jaffe reaction [17]. Serum ALT and AST activities were measured according to the method of Reitman and Frankel [18]. The Colorimetric method described by Rolinski et al. [19] was used to determine the serum total bilirubin content.

RESULTS

Table.1 shows the results of phytochemical screening of *Phyllanthus amarus* whole plant. It can be seen that the plant contains five important phytochemicals namely; tannins, saponins, flavonoids cardiac glycosides and terpenoids while phylobatannins and steroids are absent.

Table.1: Phytochemical constituents of methanolic whole plant extract of *Phyllanthus amarus*

Phytochemicals	Results
Tannins	+
Phylobatannins	-
Saponins	+
Flavonoids	+
Steroids	-
Cardiac glycosides	+
Terpenoids	+

+ = present - = not present

The results of antimicrobial sensitivity tests of *Phyllanthus amarus* extract against 14 human pathogens compared with Ciprofloxacin is shown in Table.2. The results show that the extract has bactericidal effects on *P. vulgaris, M. luteus, B. polymyxa and E. coli* with no recovery. *C. pyrogenes, B. subtilis, C sporogenes, K. pneumonia and S. aureus* were inhibited by the extract but recovered after a few hours. The other pathogens; *B. cereus, B. anthracis, B. faecelis, B. stearothermophillus* and *P. sporogenes* were not inhibited by the extract at all. Ciprofloxacin antibiotics inhibited all the 14 pathogens without recovery.

Table.2: Antimicrobial sensitivity test of *Phyllanthus amarus* on 14 human pathogens compared with Ciprofloxacin.

Tested pathogens	Zones of Inhibition (mm)			
	Phyllanthus amarus		Ciprofloxacin (standard)	
Proteus vulgaris	38	NR	28	NR
Escherichia coli	35	NR	32	NR
Micrococcus luteus	29	NR	30	NR
Bacillus polymyxa	20	NR	30	NR
Klebsiella pneumonia	14	R	30	NR
Clostridium sporogenes	14	R	28	NR
Bacillus subtilis	13	R	25	NR
Corynebacterium pyrogenes	10	R	20	NR
Staphylococcus aureus	9	R	30	NR
Bacillus cereus	0	NIL	25	NR
Bacillus anthracis	0	NIL	29	NR
Streptococcus faecelis	0	NIL	30	NR
Bacillus stearothermophillus	0	NIL	30	NR
Pseudomonas aenogenes	0	NIL	30	NR

R= Recovered; NR= No Recovery; NIL= No Inhibition

The results of MIC and MBC of the extract on the 9 affected pathogens are shown in Table.3. The results shows that *E. coli*, *P. vulgaris*, *B. polymyxa* and *M. luteus* has MBC of 6.25mg/ml. *B. subtilis* and *S. aureus* has MIC of 6.25mg/ml while *C. pyrogenes, C. sporogenes* and *K. pneumonia* has MIC of 12.5mg/ml.

Table.3: Minimum inhibitory concentration and minimum bactericidal concentration of *Phyllanthus amarus* on some human pathogens.

Pathogens	Minimum concentration of extract (mg/ml)	Effect
E. coli	6.25	Bactericidal
P. vulgaris	6.25	Bactericidal
B. polymyxa	6.25	Bactericidal
M. luteus	6.25	Bactericidal
B. subtilis	6.25	Inhibitory
S. aureus.	6.25	Inhibitory
C sporogenes	12.5	Inhibitory
K. pneumonia	12.5	Inhibitory
C. pyrogenes	12.5	Inhibitory

Figure.1 shows the growth pattern of rats administered *Phyllanthus amarus* extract. The result revealed that rats administered *Phyllanthus amarus* extract shows growth depression. The growth depression was more pronounced in rats administered 500mg/kg bw.

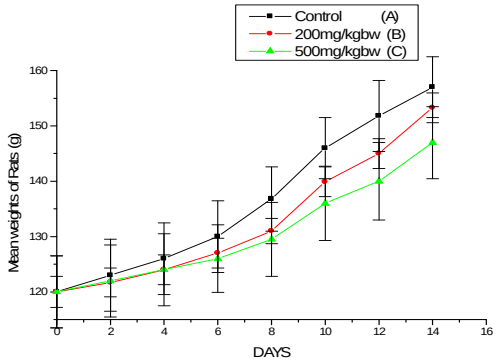

Fig.1: Growth pattern of rats administered *Phyllanthus amarus* extract

The mean kidney and liver weight index (x100) of rats administered *Phyllanthus amarus* extract is shown in Table.4. There was no significant difference between kidney and liver weight index of rats administered the extract and the control showing that the extract did not cause kidney or liver enlargement in the rats.

Table.4: Mean organ weight index (x100) of rats administered *Phyllanthus amarus* extract

Organ	Control	200mg/kg bw extract	500mg/kg bw extract
Kidney	0.63±0.02	0.66±0.01	0.67±0.01
Liver	3.15±0.20	3.27±0.07	3.30±0.10

Values are Mean ± SD of six rats. Values are not significantly different from the control at p<0.05

Table.5 shows the serum concentrations of ALT, AST, bilirubin, urea and creatinine of rats administered *Phyllanthus amarus* extract. It can be seen that the concentrations of these parameters in the serum of rats administered the extract are not significantly different from that of the control.

Table.5: Effects of *Phyllanthus amarus* extract on kidney and liver function parameters in rats serum

Test	Control	200mg/kg bw extract	500mg/kg bw extract
ALT (IU/L)	89.24±3.78	91.34±5.55	92.61±3.89
AST (IU/L)	154.70±5.55	158.29±4.94	160.18±5.32
Bilirubin (mmol/L)	4.46±0.42	4.38±0.38	4.29±0.46
Urea (mg/dl)	22.13±3.22	24.52±2.87	24.96±2.66
Creatinine (mg/dl)	3.63±0.77	3.46±0.52	3.59±0.48

Values are the Means ± SD for 6 rats in each group. Values are not significantly different from the control at p<0.05

DISCUSSION

Results obtained in this study showed that *Phyllanthus amarus* contain at least five important bioactive components of medicinal plants namely saponins, tannins, flavonoids, terpenoids and cyanogenic glycosides (Table.1). The extract also showed antimicrobial effect as it exhibited bactericidal and inhibitory activities against human pathogens with zones of inhibition comparable to that of Ciprofloxacin. This antimicrobial effect could be attributed to the bioactive phytochemicals present in the plant. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins form irreversible complexes with proline rich protein in bacteria, resulting in the inhibition of cell protein synthesis. Flavonoids disrupt microbial cell wall by forming complex with extracellular soluble proteins in the bacterial [20]. Saponins possess membrane permeabilizing properties, Inhibits histamine release in vitro and leads to vacuolization and disintegration of teguments. The current investigation confirms previous records that the plant has antibacterial properties on certain bacterial species [4].

Growth depression was recorded in rats administered the extract compared to the control. The observed growth depression may arise due to poor appetite, mal absorption, intestinal irritation, poor food efficiency, excessive faecal formation and nutrient loss caused by the extract. It might also result due to increase degradation of structural proteins in the muscle of rats which eventually causes weight loss [21]. Tanins present in the extract has also been implicated in causing intestinal irritation and mal absorption in rats when present in high concentration [22].

Administration of *Phyllanthus amarus* to rats has no significant effect of the liver and kidney weight compared with the control as observed in Table 4. This is an indication that the extract did not induce organ enlargement or shrinking in the rats. These results

indicate a non-toxic effect of the extract on these organs.

Results in Table 4 revealed that the extract did not alter urea, creatinine, ALT, AST and bilirubin concentration in the serum of the rats significantly. This is an indication that the extract has no adverse effects on the metabolism and excretion of these metabolites/enzymes in rats. Creatinine and urea are markers of kidney function [23]. They are waste products of metabolism, found in the liver and conveyed in blood to the kidney for excretion. Healthy kidneys remove urea and creatinine out of the blood into urine. If a person's kidneys are not working well, these metabolites remains in the blood. Altered creatinine levels can be used as an indicator of kidney dysfunction, or may be associated with other conditions that result in decreased renal blood flow [24]. Increase in serum creatinine has also been reported to arise from intrinsic renal lesions, decreased perfusion of the kidney, or obstruction of lower urinary tract malaria infection [25].

Serum urea has been reported to increase in acute and chronic intrinsic renal disease and also when there is decreased effective circulating blood volume with decreased renal perfusion [25, 26]. This result suggests that the extract has nephron protective properties.

The observed non alteration in serum ALT and AST in the rats indicates its hepatoprotective potential. ALT and AST are present in high concentration in the liver where they play key roles in transamination. The levels of these enzymes are raised in liver necrosis and other conditions that promote abnormal liver cell membrane permeability [27]. In mild liver injury, only cytoplasmic enzymes are released while severe liver damage causes release of mitochondrial enzymes as well [27].

CONCLUSION

Results obtained in this study showed that methanolic extract of *Phyllanthus amarus* contains active phytochemicals with antimicrobial effects against some microorganisms that cause diseases in humans. The results also show that the plant is neither nephrotoxic nor hepatotoxic as it does not show any significant change in the kidney and liver function indicators in rats. We hereby conclude that the use of *Phyllanthus amarus* as herbal remedy for the treatment of various ailment is safe.

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