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Antimicrobial, phytochemical and pharmacological properties of *Phyllanthus niruri* linn

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Abstract

Introduction: *Phyllanthus niruri* is a common herb widely used in home remedies against infectious agents. This study unveils the antimicrobial and therapeutic potentials of *P. niruri* against *Escherichia coli* infection.

Methods: Ethanol and water extracts of the plant were prepared and investigated for their antimicrobial activity using the agar well diffusion method against eleven clinical isolates. The *in vivo* study was conducted on albino rats, infected and subsequently treated.

Results: The observation showed Ciprofloxacin with the highest (41 mm) sensitivity against *P. mirabilis;* and lowest (20 mm) against *S. flexneri, E. coli, K. pneumoniae,* and *S. aureus.* Ketoconazole at 100 mg/mL concentration revealed antifungal sensitivity ranging from 2 to 15 mm. The extracts showed better sensitivity against the bacteria (2 to 24) mm when compared with the fungi species (2 to 12 mm). *Phyllanthus niruri* extract demonstrated a minimum inhibitory concentration of 25 to 100 mg/mL The albino rats of weight ranging from 83 to 105 g were administered with *P. niruri* ethanol extract but indicated no toxicity at 1500 mg dose. The packed cell volume, red cell count, total leucocyte count, and serum enzymes of the tested rats were within the normal range. The healing effect was dose-dependence and most effective from 1200 mg/kg to 1500 mg/kg body weight. *P. niruri* extract produced some level of antimicrobial activity both *in vitro* and *in vivo.*

Conclusion: Extract from *Phyllanthus niruri* is effective *in vitro* and *in vivo* against *Escherichia coli* infection. It has no ill effect on the blood circulatory system, liver, and kidney. The bioactive agent present in the extract has proven health benefits, and can be administered as supplement. However, more studies on its chronic toxicity are required.

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Propriétés antimicrobiennes, phytochimiques et pharmacologiques du *phyllanthusniruri* linn

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Résumé

Objectif de l'étude: *Phyllanthusniruri* est une herbe commune largement utilisée dans les remèdes de foyer contre les agents infectieux. Cette étude dévoile les potentiels antimicrobiens et thérapeutiques de *P. niruri* contre l'infection *à Escherichia coli.*

Méthode de l'étude: Des extraits d'éthanol et d'eau de la plante ont été préparés et étudiés pour leur activité antimicrobienne en utilisant la méthode de diffusion en puits d'agar contre onze isolats cliniques. L'étude *in vivo* a été menée sur des rats albinos, infectés puis traités.

Résultat de l'étude: L'observation a montré la ciprofloxacine avec la sensibilité la plus élevée (41 mm) contre *P. mirabilis ; et* le plus bas (20 mm) contre *S. flexneri , E. coli, K. pneumoniae et S. aureus* . Le kétoconazole à une concentration de 100 mg/mL a révélé une sensibilité antifongique allant de 2 à 15 mm. Les extraits ont montré une meilleure sensibilité contre les bactéries (2 à 24) mm par rapport aux espèces de champignons (2 à 12 mm). L'extrait *de Phyllanthusniruri* a démontré une concentration minimale inhibitrice de 25 à 100 mg/mL. Les rats albinos de poids allant de 83 à 105 g ont reçu de l'extrait d'éthanol de *P. niruri* mais n'ont indiqué aucune toxicité à la dose de 1500 mg. L'hématocrite, le nombre de globules rouges, le nombre total de leucocytes et les enzymes sériques des rats testés se situaient dans la plage normale. L'effet curatif était dose-dépendant et le plus efficace de 1200 mg/kg à 1500 mg/kg de poids corporel. L'extrait *de P. niruri* a produit un certain niveau d'activité antimicrobienne à la fois *in vitro* et *in vivo.* L'agent bioactif présent dans l'extrait a des effets bénéfiques prouvés sur la santé et peut être administré en complément.

Déclaration importante : L'extrait de *Phyllanthusniruri* est efficace *in vitro* et *in vivo* contre l'infection *par Escherichia coli.* Il n'a aucun effet néfaste sur le système circulatoire sanguin, le foie et les reins. Cependant, d'autres études sur sa toxicité chronique sont nécessaires.

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INTRODUCTION

Complications due to drug-resistant and epidemics caused by microbial organisms of unknown origins such as bacteria are still a leading cause of death worldwide. The situation has called for improved strategies and has led to the continuous search for antimicrobials and drug development from natural sources. This approach will enhance the treatment and prevention of life-threatening diseases (1). Assessment of plants for their healing potentials and toxicology could give knowledge and data of how plants and their extracts can be of maximal benefit for the treatment. Some researchers have reported the usefulness of some *Phyllanthus* species in medicine. *Phyllanthus* contains secondary metabolites that have been employed in traditional cures (2, 3). Phyllantin, flavonoids, alkaloids, tannins, terpenes, and sterols are some of the biochemical components inherent in *Phyllanthus*. Extracts from this herb possess anti-viral and anti-hypertensive action (4). *Phyllanthus niruri* L. (Euphorbiaceae) is known for its ability to protect the liver (5). The antimicrobial properties and other pharmaceutical principles have not been fully exhausted. This study evaluates the antibacterial, antifungal, and therapeutic effects of the plant against *Escherichia coli*.Therefore the worldwide use of *Phyllanthus* demands the need to estimate the pharmacological efficacy and safety of the extract.

MATERIALS AND METHODS

Source of test organisms

Clinical isolates were obtained from the Microbiology Department of Ladoke Akintola University of Technology Teaching Hospital, Osogbo. Each microbial isolate was confirmed using cultural characteristics and standard biochemical tests. The bacterial isolates were *Staphylococcus aureus, Salmonella typhimirum, Escherichia coli, Shigella flexneri, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, and Streptococcus viridian.* The fungi isolates were *Candida albicans, Aspergillus flavus, and Aspergillus niger.*

Preparation of extracts from *P. niruri*

Fresh plant was harvested from various places in Osogbo, South West Nigeria. A fresh sample of the plant was identified and authenticated by a Botanist at the Botany department, Ife Herbarium, Obafemi Awolowo University, Ile-Ife, Osun State (Herbarium number: IFE 17292). The whole plant including the stem, root, leaves together with the seeds were carefully picked, washed and air dried at 28 °C. Aqueous and ethanol extract of the whole plant was obtained using the cold extraction method at ratio 1: 10 of ground powder to extracting solution. It was soaked for 48 h at 28 °C. The supernatant was filtered using muslin cloth and filtrate concentrated *in vacuo* in a rotary evaporator, then evaporated to dryness in a clean laboratory oven at 40 °C.

Evaluation of extracts for *in vitro* antimicrobial potentials

Agar plate well diffusion method described by Mounyr*et al*., (6) was employed to evaluate the antibacterial assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts. Five micrograms (5µg) ciprofloxacin and 100 mg/L ketoconazole were used as control for antibacterial and antifungal respectively.

Analysis of phytochemicals inherent in the extracts

Ethanol and water extracts of *P. niruri* were analyzed for their phytochemicals: cardiac glycosides, steroids, tannins, flavonoids, and terpenes (7, 8).

Evaluation of extracts for *in vitro* antimicrobial potentials

The agar - plate well diffusion method (6) was employed to evaluate the plant extracts for antibacterial assay. Minimum inhibitory concentration (MIC) and MBC of ethanol extract were also determined as described by Mounyr *et al*. (6). Standard antibacterial and antifungal used were 5µg ciprofloxacin and 100 mg/mL ketoconazole respectively.

Handling of animals and experimental design

Thirty (30) healthy Wistar albino rats of weight 82.0 g to 92.2 g were purchased from the animal house of the College of Health Sciences, Obafemi Awolowo University, Ile- Ife, Osun State in Nigeria. The animals were housed in plastic cages and fed with commercially produced standard rat pellets once a day and clean drinking water. The beddings were laid with wood powder and changed daily, feeding utensils, troughs, and the environment were maintained clean. The room temperature was at 25 °C and the light was 12 h light and 12 h darkness. Animal maintenance and handling were humane and followed the National Institute of Health (NIH) animal care guidelines (9).

The experiment was conducted in a Completely Randomized Design (CRD). The Wistar rats were distributed into 6 groups (n = 5) with an equal number of sexes in each group, and the sample size was determined in advance. They were allowed to acclimatize in 7 days before the commencement of the experiment. Group CI N was the normal control, not inoculated, and received feed and clean water. Group CII P was a positive control, inoculated and administered with a standard antibacterial (Ciprofloxacin) group 2 to group 5 were inoculated and administered with 300 mg, 600 mg, 1200 mg, and 1500 mg doses of *P. niruri* ethanol extract respectively. The extract was administered via oral route.

Inoculation and Evaluation of Graded doses of *P. niruri* ethanol extract on albino rats.

Escherichia coli obtained from the clinical sample was inoculated into physiological saline. The suspension was incubated at 37 °C for 24 h to obtain a bacterial suspension equivalent to 1×10^6 CFU. A 0.30 ml of the actively growing bacterial suspension was administered via an oral route. Studies showed that LD50 of ethanol extract of *P. niruri* is greater than 5 kg per body weight (10) After 3 days, groups 2 to 5 were administered with a graded dose of *P. niruri* ethanol extract (300, 600, 1200, and 1500 mg/kg body weight) respectively for 14 days. A Ciprofloxacin (Cip) tablet, (brand: Fidson) was obtained from a registered Pharmaceutical shop. A sterile suspension of 1.34 mg/ml Cip was prepared and administered to the positive control group for 7 days.

Microbiological culture of faecal sample obtained from the rats.

The faecal sample of the rats was collected in a clean universal bottle after three days of bacterial inoculation, 7 days and 14 days of administration of the ethanol extract. The aseptic condition was maintained throughout the experiment. A 0.5 g of the stool sample was inoculated in Selenite F, incubated at 37°C for 24 h then subcultured on MacConkey and Eosin Methylene Blue agar using standard operating procedures (11).

Biochemical and haematological analysis of blood samples obtained from the treated rats

The Wistar rats were starved for 12 h, sacrificed by the cervical dislocation method, and blood was withdrawn from the heart under an aseptic condition. The needle was removed and blood was released in a clean dry plain bottle and an EDTA (Ethylene diaminetetraacetic acid) bottle. The blood sample in a clean dry plain bottle was allowed to clot and spun at 3000 rpm for 10 minutes. The serum was obtained, labelled, and used to conduct biochemical analysis for Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), and Creatinine using enzyme maker kits from Randox (11). The blood sample released in an Ethylene diaminetetraacetic acid (EDTA) bottle was gently mixed on a haematology roller and analysed for Packed cell volume (PCV), Leucocyte total count, Leucocyte differential count, Neutrophil (N), Lymphocyte (L), Eosinophil (E), Monocyte (M), and Basophil (B) in percentage as described by Cheesebrough (11).

Statistical Data

The results of replicates were pooled and expressed as Mean ± Standard error of mean. Data obtained were subjected to a two-way analysis of variance and treatment means were compared using Duncan's new multiple-range tests with the aid of Statistical Package for Social Sciences (SPSS) software, version 17. Differences between treated groups were considered significant at $P \leq 0.05$.

RESULTS

The aqueous and ethanol extracts of *Phyllanthus niruri* were bitter and acidic with a pH of 6. Quantitatively ethanol extract of *Phyllanthus niruri* revealed cardiac glycosides as the highest phytochemical compound, followed by steroids, tannin, flavonoids, and terpenes in decreasing order. (Table 1) In the aqueous extract cardiac glycosides was also of the highest concentration, followed by flavonoids, then tannins in decreasing order and the least was steroid; terpenes were not present.

In Table 2, the standard antibiotics (5 µg ciprofloxacin) showed antibacterial activity with an inhibitory zone ranging from 20 to 41 mm and no significant resistance against *P. aerugenosa*. Aqueous extract of *P. niruri*.demonstrated a zone of inhibition ranging from 2 to 18 mm and no resistance to *S. viridian and P. aerugenosa* (Table 2). The ethanol extract of *P. niruri* showed an inhibitory zone ranging from 2 to 24 mm but no significant resistance against *S. viridian* only (Table 2). The ethanol extract of *P. niruri*. demonstrated better antibacterial resistance when compared with the aqueous extract $P \leq 0.05$.

Table 3 showed the highest antifungal activity in 100 mg/ml ketoconazole with the zone

of inhibition ranging from 2 to 15 mm. Aqueous extract of *P. niruri* demonstrated antifungal sensitivity ranging from 2 to 12 mm while ethanol extract showed no significant antifungal sensitivity to any of the fungal isolates (Table 3).

The ethanol extract of *P. niruri* showed MIC of 25 to 100 mg/ml and MMC (minimum microbicidal concentration) of 30 to 160 mg/ml. The Minimum inhibitory concentration demonstrated against *A. flavus* and *A. niger* were 50 and 100 mg/ml respectively while their minimum fungicidal concentration (MFC) was zero. *In vivo* testing was conducted on Wistar rats and Table 4 showed reduced activity and rough fur after three days (day 9) of exposure to *E. coli* infection; followed by improved activity and appetite for food from day 16 to day 23. The normal and positive control, groups 4 and 5 demonstrated a scanty growth while groups 2 and 3 showed a significant growth on day 23 (Table 5). The susceptibility of *E. coli* to the extract was dose-dependent.

The haematology parameters of the experimental animals revealed no significant variation in the packed cell volume, red cell count, leucocyte count, lymphocyte, eosinophil, monocyte, and basophil $P \leq 0.05$ (Table 6). Lymphocyte was of a higher percentage than neutrophil in test and control, the exception was group 4.

The positive control showed the lowest values of the ALP, ALT, and Creatinine (45.33±3.48 µ/L, 28±6.56 µ/L, 88±7 µMOL/L); the negative control displayed the highest Creatinine level (Table 7). In the test groups, the ALP, ALT, and AST were at the least concentration in group 5. However, there was no statistical difference between the test and controls $P \leq 0.05$

DISCUSSION

Extracts of *phyllanthus* is extensively used in traditional medicine and was reported to have antibacterial and antiviral activity (12).

Ethanol is revealed as a better extractive solvent of its phytochemicals for better yield of the biochemical compounds. This is because ethanol is generally able to dissolve multivariable types of compounds (13).

Tannins, an effective compound present in moderate amount in *P. niruri.* are known antimicrobial biomolecules (14). Flavonoids are antioxidant and possess the ability to complex with bacteria cell wall (15). Moreover, Cardiac glycosides has some beneficiary effects but are toxic at a high level (16). Terpenes, has lipophylic character and are active against a wide variety of microorganisms (17) while steroids have anti-inflammatory action. The results of this study corroborate previous workers' findings that the plant contains antimicrobial substances (18). *Phyllanthus niruri* is a source of bioactive substances of broad-spectrum activity because of its sensitivity against both bacteria and fungi. However, the bioactive compounds are more active against gram- negative than gram- positive bacteria as the aqueous extract of the plant showed (zone of inhibition) The result supports the findings of Komuraiah *et al.,* (19) who reported *Phyllanthus amarus* is an effective inhibitor of both gram- negative- and gram-positive bacteria. The better sensitivity of the plant extract against gram- negative species could be due to the thin peptidoglycan layer of gram- negative bacteria that is more permeable to antibacterial (20). The fungi species used in this study showed 33.3 % sensitivity to the aqueous extract of *P. niruri L.* at Z inhibition ≥ 10 indicating that the bioactive agents in the extract were relatively more effective against the bacterial species than the fungal species.

The low MIC and MBC obtained are indications of its potency against bacterial infections however, the zero Minimum Fungicidal Concentration (MFC) of *P. niruri* against *Aspergillus* species is suggestive of 100 % resistance which means when subjected to favorable conditions the fungi could thrive and replicate.

In the *in vivo* studies, the initial reduction in the average body weight was due to the *Escherichia coli* (*E. coli*) infection in the rats. A prominent symptom of *E. coli* infection is frequent passage of stool which oftentimes leads to a drop in body weight. The improvement in the weight of the Wistar albino rats after treatment indicates increased feed conversion efficiency (FCE). This development could be the result of recovery from the infection arising from restoration of the physiological activities of the rat system. The improvement in the health status of the rats could also be traced to the fact that chemical and biochemical constituents of plants which include carbohydrates, vitamins, protein, alkaloid, fats and oil and minerals might supply the body cells with energy and replace worn- out tissues.

The bioactive agents present in *P. niruri* proved effective *in vitro* and *in vivo* against *E. coli* infection. The weight gain of the rats reduced as the dose increased. This indicates that irrational consumption of the plant could be potentially

harmful. The bacterial load of *E. coli* in the test animals reduced gradually as the doses administered increased and after two weeks of treatment with the extract (1200 mg to 1500 mg) the bacterial count became insignificant.

Red Blood Cell count (RBC) and Packed Cell Volume (PCV) which are important parameter for blood health status is indicative of normal erythropoiesis. Flavonoids, an essential compound present in *P. niruri* L. are an antioxidant element that ensures healthy blood circulation, strengthens the capillary wall and reduces blood cholesterol level. The blood leucocytes are the body soldiers that are involved in eliciting an immune response against the infectious agent. Leucocytes were within the normal range in the test animal, likewise neutrophil which indicates that the infection was inhibited

As a measure of liver function test, the AST (Aspartate amino transaminase), ALT (Alanine amino transaminase), and ALP (Alkaline phosphatase) were assessed in *P. niruri* treated rats. An elevation of these enzymes is suggestive of liver damage (21). The administration of *P. niruri* caused a decline in the liver enzymes. This suggests that *P. niruri* may hold a positive impact on the liver cells and this corroborates the holdings of Verma *et al*., (22) who recorded that the extract of *P. niruri* is hepatoprotective.

Serum urea and creatinine are labels of renal positions. High creatinine level is a pointer of impairment in renal filtration but low throughout the treatments. Howbeit, Eweka and Enogieru (23) noted some necrosis in the anatomical structures of the gastro intestinal tract (GIT) following 28 days of oral administration of *P. amarus.* The variation observed in the result may be due to differences in the duration of administering the extract.

CONCLUSION

In conclusion, *Phyllanthus niruri* has potent bioactive compounds against *Escherichia coli* infection. The administration of this extract up to 1500 mg dose has no deleterious effect on the liver and the kidney However, exploratory studies on *P. niruri is required* to determine the active compound (s) responsible for the antimicrobial effect demonstrated and evaluate its toxicity profile.

Conflict of Interest: The authors state no conflict of interest

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Table 1: Quantitative analysis of the phytochemical constituents of aqueous and ethanol extracts of *P. niruri*.

Phytochemicals	Aqueous extract (mg/g)	Ethanol extract (mg/g)
Tannins	11.30±0.02 [a]	13.49±0.05 [b]
Flavonoids	11.59±0.04 [a]	12.60±0.01 [a]
Steroid	8.08±0.30 [a]	15.01±0.01 [b]
Terpenes	0.00±0.00 [a]	10.68±0.01 [b]
Cardiac Glycosides	25.45±0.02 [a]	26.46±0.33 [b]

Key: Values are means ± Standard error of mean of 2 replicates of the phytochemical constituents.
Means with different superscript on the same row is significant

Table 2: Antibacterial sensitivity of aqueous and ethanol extract of *P. niruri*.

Antimicrobials (mg/mL) /ZI(mm)	*P. mirabilis*	*E. coli*	*S. viridian*	*P. aeruginosa*	*K. pneumonia*	*S. aureus*	*S. typhi*	*S. flexmeri*
AE30	10.67±0.67^{b}	07.00±5.80^{bc}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	08.67±0.67^{c}	02.00±0.00^{b}	06.33±0.33^{b}
AE50	16.67±6.67^{cd}	07.00±5.80^{bc}	00.00±0.00^{a}	00.00±0.00^{a}	02.00±0.00^{a}	08.67±0.67^{c}	02.00±0.00^{b}	06.00±0.00^{b}
AE100	18.00±0.00^{de}	18.00±4.00^{cd}	00.00±0.00^{a}	00.00±0.00^{a}	04.00±0.00^{b}	10.00±2.00^{c}	08.00±0.00^{d}	10.00±0.00^{d}
EE30	11.67±0.33^{bc}	04.00±0.00^{ab}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	04.00±0.00^{b}	00.00±0.00^{a}	07.67±0.33^{c}
EE50	23.33±4.81^{c}	10.67±3.06^{c}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	04.00±0.00^{b}	00.00±0.00^{a}	10.00±0.00^{d}
EE100	24.00±0.00^{f}	18.67±2.00^{de}	00.00±0.00^{a}	02.00±0.00^{a}	04.00±0.00^{b}	04.67±0.60^{b}	06.00±0.00^{c}	07.67±0.00^{c}
C1N	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}
C1P	41.33±0.00^{g}	20.00±0.00^{e}	21.67±0.33^{b}	00.00±0.00^{a}	20.00±1.50^{c}	20.00±0.00^{d}	28.00±0.00^{e}	19.67±0.33^{e}

Key: Values are the Means ± Standard error of means of the 3 replicates of the zone of inhibition. Means with different superscript on the same column is significant P = 0.05; ZI = Zone of inhibition; AE30 – aqueous extract at 30 mg/mL; AE50= aqueous extract at 50 mg/mL; AE100= aqueous extract at 100 mg/mL.; EE30 - ethanol extract at 30 mg/mL.; EE50= ethanol extract at 50 mg/mL.; EE100= ethanol extract at 100 mg/mL.; C 1N – negative control; C 1P - positive control

Table 3: Antifungal activity of aqueous and ethanol extracts of *P. niruri* .

Antimicrobials (mg/mL) /ZI(mm)	*A. flavus*	*A. niger*	*C. albicans*
AE30	02.00±0.00^{b}	07.67±0.00^{c}	00.00±0.00^{a}
AE50	12.00±0.00^{d}	08.00±0.00^{d}	02.00±0.00^{b}
AE100	00.00±0.00^{a}	08.00±0.00^{d}	08.00±0.00^{c}
EE30	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}
EE50	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}
EE100	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}
C1N	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}
CIIP	06.00±0.00^{c}	02.00±0.00^{b}	15.00±0.00^{d}

Key: Values are the means ± Standard error of means of the 3 replicates of the zone of inhibition.

Means with different superscript on the same vertical column is significant P = 0.05;

ZI = Zone of inhibition; AE30 – aqueous extract at 30 mg/mL; AE50= aqueous extract at 50 mg/mL;

AE100= aqueous extract at 100 mg/mL; EE30 - ethanol extract at 30 mg/mL; EE50= ethanol extract at 50 mg/mL;

EE100= ethanol extract at 100 mg/mL; C 1N – negative control; C 11P - positive control

Table 4: Clinical appearance of infected albino rat during treatment

Days	CI N	CII P	G2	G3	G4	G5
D 6	Fine fur; very active	Fine fur; very active	Fine fur; very active	Fine fur; very active	Fine fur; very active	Fine fur; very active
D 9	Fine fur; very active	Activity reduced; rough fur	Activity reduced; rough fur	Activity reduced; rough fur	Activity reduced; rough fur	Activity reduced; rough fur
D 16	Fine fur; very active	Very active; Few insects the cage	Activity improved slightly; wet faeces	Activity improved; wet faeces	Activity improved; wet faeces	Activity improved; wet faeces
D 23	Fine fur; very active	active; Improved fur	Increased appetite for food	Increased appetite for food	Increased appetite for food	Increased appetite for food

Key:CI N = normal control, CII P = positive control, G2 = group 2, administered with 300 mg extract,

G3 = group 3, administered with 600 mg extract, G4 = group 4, administered with 1200 mg extract,

G5 = group 5, administered with 1500 mg extract

Table 5: Effect of *Phyllanthusniruri* on *Escherichia coli*

Day	Normal control	Positive Control	G2	G3	G4	G5
9	±	+++	+++	+++	+++	+++
16	±	±	+++	++	++	+
23	±	±	++	+	±	±

Key: ± scanty growth; + few growth; ++ moderate growth; +++ heavy growth,

G2 = group 2, administered with 300 mg extract, G3 = group 3, administered with 600 mg extract,

G4 = group 4, administered with 1200 mg extract, G5 = group 5, administered with 1500 mg extract

Table 6: Effect of ethanol extract of *P. niruri* on haematology parameters of albino rats

Group	Sex	PCV (%)	RBC (10^{12}/L)	WBC (10^{9}/L)	N (%)	L (%)	E (%)	M (%)	B (%)
G2	1.50±0.29^{a}	31.50±3.30^{a}	03.85±0.30^{a}	07.90±1.38^{a}	30.75±2.80^{a}	68.75±3.20^{b}	0.50±0.50^{a}	0.00±0.00^{a}	0.00±0.00^{a}
G3	1.25±0.25^{a}	31.75±2.14^{a}	03.95±0.24^{a}	09.63±2.45^{a}	41.75±3.30^{a}	58.25±3.30^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
G4	1.75±0.25^{a}	32.75±1.89^{a}	04.08±0.22^{a}	12.43±3.21^{a}	54.50±3.80^{b}	45.50±3.80^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
G5	1.50±0.28^{a}	37.50±2.99^{a}	04.60±0.32^{a}	10.23±2.80^{a}	42.75±4.31^{a}	57.00±4.42^{ab}	0.00±0.00^{a}	0.25±0.20^{a}	0.25±0.25^{a}
CI N	1.00±0.00^{a}	35.00±2.55^{a}	04.18±0.23^{a}	11.72±3.26^{a}	36.00±1.30^{a}	63.40±1.44^{b}	4.00±0.00^{a}	0.60±0.40^{a}	0.00±0.00^{a}
CII P	1.60±0.25^{a}	32.20±1.66^{a}	04.02±0.14^{a}	05.90±1.12^{a}	35.40±5.71^{a}	64.40±5.78^{b}	0.00±0.00^{a}	0.20 ±0.20^{a}	0.00±0.00^{a}

Key: Values are the Means ± Standard error of means of 5 replicates of the haematology parameters evaluated. Means with different superscript on the same vertical column is significant P = 0.05; RBC - red blood cells; WBC - white blood cells total count; N – neutrophil; L – lymphocyte; E – eosinophil; M – monocyte; B – basophil, CI N – normal control, CII P – positive control, G2 = group 2, administered with 300 mg extract, G3 = group 3, administered with 600 mg extract, G4 = group 4, administered with 1200 mg extract, G5 = group 5, administered with 1500 mg extract

Table 7: Effect of ethanol extract on biochemical parameters of Wistar albino rat

	SEX	ALP µ/L	ALT µ/L	AST µ/L	CREATµ MOL/L
Control 1 N	1.00±0.00[a]	98.00±25.16[a]	58.00±15.95[a]	177.33±54.82[a]	158.00±36.51[a]
Control 11 P	1.60±0.25 [a]	45.33±3.48[a]	28.00±6.56[a]	128.67±30.18[a]	88.00±7.00[a]
G 2 300 mg	1.50±0.29 [a]	88.75±24.56[a]	62.00±16.53[a]	184.00±59.23[a]	118.50±31.52[a]
G 3 600 mg	1.25±0.25 [a]	82.00±43.00[a]	59.00±29.00[a]	187.00±83.00 [a]	129.00±72.0[a]
G 4 1200 mg	1.75±0.25 [a]	59.67±13.17[a]	34.67±8.82[a]	108.00±12.81 [a]	99.00±13.75[a]
G 5 1500 mg	1.50±0.25 [a]	46.33±0.33[a]	28.33±4.70[a]	95.00±17.94 [a]	104.00±8.19[a]

Key: Values are the Means ± Standard error of means of 5 replicates of the biochemical parameters evaluated.

Means with different superscript on the same vertical column is significant P = 0.05;

Sex - male and female; ALP - alkaline phosphatase; ALT = alanine aminotransaminase; AST = aspartate aminotransaminase; Control I N – normal control; Control II P – positive control; G2 = group 2, administered with 300 mg extract, G3 = group 3, administered with 600 mg extract, G4 = group 4, administered with 1200 mg extract, G5 = group 5, administered with 1500 mg extract