*Original Research Article*

# Determination of Phytochemical composition and the Effects of Ethanol extract of *Phyllanthus urinaria* on the Liver and Kidney function parameters in Paracetamol-administered Albino Rat models


## ABSTRACT

The current study was aimed at determining the phytochemical composition and the effects of *Phyllantus urinaria*. Ethanol extract on the liver and kidneys by using albino rats as the animal models. All the analyses were carried out following standard laboratory methods and procedures. A total of fifteen rats were randomly distributed into three groups of five rats in each group where group one served as the control while the test groups comprise of 1000mg/kg body weight (b.w) and 500mg/kg b.w administered daily for fourteen days and followed by administration of a high dose of paracetamol to challenge the system toxicologically. This was deliberately done to note whether the extract having administered for many days will protect the liver and kidneys against the paracetamol by determining the serum biomarkers. The results showed the presence of saponins (8.84 g/100g), tannins (1.32 g/100g), phenols (0.11 g/100g), flavonoids (0.512 g/100g) and alkaloids (0.038 g/100g) while cardiac glycosides, Resins, terpenoids and steroids were not detected. The activity of ALP was significantly ($p < 0.05$) higher in both the 1000mg/kg b.w and 500mg/kg/b.w. There was no significant ($p>0.05$) decrease in ALT activity of 1000mg/kg b.w but at 500mg/kg b.w. the activity of ALT significant ($p>0.05$) increased when compared with the control. There was a significant ($p<0.05$) decrease in AST activity of 1000mg/kg b.w and 500mg/kg b.w when compared with the control. Bilirubin concentration significantly ($p< 0.05$) decreased at 1000mg/kg b.w and non-significanlly ($p>0.05$) increased at 500mg/kg b.w. ALB significantly ($p > 0.05$) increased at 1000mg/kg b.w and 500mg/kg b.w. a non-significant decrease in Serum HCO3- in both 1000mg/kg/b.w and 500mg/kg b.w. UREA non-significantly decreased at both 1000mg/kg b.w and 500mg/kg b.w. Creatinine significantly increasde at both 1000mg/kg b.w and 500mg/kg b.w concentration when compared to the control. $Na^+$ showed a non-significant increase at 1000mg/kg b.w and 500mg/kg b.w when compared to the control. The extract may not have protected the liver and kidneys against paracetamol despite the presence of some phytochemicals, a histological examination of the liver and kidneys is therefore recommended so as to have a clearer picture of the effects observed.


**Keywords:** Bioactive compounds, Hepatocytes, Nephrology, Biomarkers, Toxicology.


## INTRODUCTION

There are many factors that may course Liver injury which include viruses, drugs and chemicals, leading to serious toxicological problem (De et al., 2004;Hoek et al., 2002;Jaeschke et al., 2002). Liver damage may be associated with metabolic and synthetic dysfunctions which if not properly treated or not treated or left untreated can lead to fatal complications (Orhan et al., 2007). For experimental purposes many different substances such as alcohol, paracetamol, $CCL_4$ are often used to induce liver toxicity in animal models. Although CCl4-induced acute liver injury is said to be the best characterized system of xenobiotic-induced hepatotoxicity and a common screening model for to evaluate the hepatoprotective

potential of drugs and other chemical agents (Jain et al., 2011), we opted for paracetamol because it was more easily accessible. The pathogenesis of the damage is multidimensional (McGregor and Lang, 1996) involving propagation of a chain of free radicals and chain reactions, leading to lipid peroxidation and destruction of cell membranes, and inducing the inflammatory response of the body which attack and kill normal cells (Edwards et al., 1993, Recknagel et al., 1989).

The term "Nephrotoxicity" refers generally to the harmful effects of substances on the kidneys. It occurs when the kidneys are exposed to toxins thereby subjecting it to detrimental effects. One way to prevent firther complications is by administration of detoxifying agents to avoid kidney damage or kidney failure. In the event where the toxic effect leads to kidney failure, it becomes impossible for the body to pass out urine. Accumulation of urine in the body further damages the kidneys. One of the markers of Nephrotoxicity is a temporary elevation of serum (BUN and/or creatinine).

Paracetamol is a Non-Steroidal Anti-Inflammatory Drug (NSAID) that is widely used to treat pain and fever. At therapeutic doses, paracetamol is well tolerated and has lower incidences of adverse effects compared to other NSAID such as aspirin (Graham *et al*, 2005). Due to its safety paracetamol is widely misused for the relief of pain.

The plant "*Phyllanthus urinaria"* is a member of the family of flowering plants Phyllanthaceae and have over 1000 species vastly distributed in many parts of the globe (Mao *et al*., 2016). The species of this genus comprising of trees, herbs and shrubs that are pharmacologically valuable as they contain many different bioactive compounds (Calixto *et al*., 1998; Mao *et al*., 2016). It is of interest to note that crude extracts obtained from different species of Phyllanthus have inhibitory effects against the hepatitis B virus (HBV). Previous reviews broadly highlight the biological activities of Phyllanthus species, mostly from P. amurus Schum. and Thonn., P. emblica L. or P. niruri L. (Calixto *et al.,* 1998; Mao *et al.,* 2016; Kaur *et al.,* 2017; Tewari *et al.,* 2017; Yadav *et al.,* 2017). There is also the need to do a specific and detailed review of *P. urinaria*. So as to provide scientific proof for its ethnopharmacological and traditional uses although recent scientific studies focus on its chemical constituents and their biological properties. The

current study was aimed at determining the phytochemical contents of *Phyllantus urinaria* extract and to determine its effect on the Liver and Kidney function indices.

Figure 1: *Phyllantus urinaria*

## MATERIALS AND METHOD

### Materials

**Plant material:** Fresh whole plants of *P. Urinaria* were collected from its natural habitat at a Angwan Lambu area in Keffi Local Government Area, Nasarawa State, Nigeria. The local traditional medicinal practitioners assisted in identifying the plant and was confirmed in the Department of Plant Science and Biotechnology, Nasarawa State University, Keffi, Nigeria.

**Experimental Animals:** Fifteen adult Albino rats weighing 100-210 g were used in the study. The rats were housed in cages of 5 rats each and allowed acclimatization to laboratory status for one week before the experiments commenced. The rats were maintained at room temperature with a 12h light/12h dark cycle and allowed access to feed and water *ad libitum*.

### Chemicals and Reagents

All the chemicals and reagents used were of analytical grade and products of Sigma Aldrich. They include: Carbon tetra chloride ($CCL_4$), Potassium ferricynide, Hydrogen chloride, Distilled water, Ammonia ($NH_3$), Concentrated tetraoxosulphate vi acid (conc. $H_2SO_4$), Acetic Anhydride, Wagner's

Reagent/ Mayer's reagent, Sodium Hydroxide ($N_aOH$), Chloroform, Glacial acetic acid, Ammonium hydroxide ($NH_4OH$), Acetic acid, Ethanol, Sodium chloride (NaCl).

**Methods**

**Preparation of plant material:** The whole plant materials were air dried at room temperature for two weeks until they were thoroughly dried. They were then ground into fine powder using an electric blender.

**Extraction:** The powdered sample material weighing 400g was soaked in one liter of ethanol. The highly polar solvent have been known to give a high yield of bioactive compounds due to its high ability to dissolve a wide range of compounds, at the same time giving rise to extract that could be concentrated at a lower temperature range for 72 hours with occasional swirling to facilitate the extraction process. The mixture was then filtered using muslin clothe to remove the coarse particles followed by filtration with filter paper to obtain a clear filtrate. The filtrate was concentrated using a rotary evaporator at 55°C and stored in stoppered containers at 4°C until use.

**Experimental Design**

The study was carried out using fifteen (15) adult albino rats weighing between 100-210g. They were randomly distributed into three (3) groups of five (5) rats in each group and administered different substances thus; group 1 serves as the normal control, group two was administered 1000mg/kg body weight (b.w) of the extract, group 3 was administered 500mg/kg b.w of the extract for fourteen days. After that the rats were administered 500mg/kg b.w of paracetamol on day fourteen to challenge the liver and the kidneys through intoxication. The blood samples were then collected to analyze for liver and kidney function parameters.

**Qualitative Phytochemical Screening**

Preliminary qualitative phytochemical screening of the ethanol bark extract of *A. grandiflora* was carried out to determine the class of secondary metabolites present using standard procedure according to

Harbone (1973). Active principles tested included tannins, saponins, alkaloids, flavonoids, glycosides, phenols, terpenoids, cardiac glycosides, anthraquinones, steroids, phlobatannins and anthracyanine, as many of them have been known to be associated with analgesic and anti-inflammatory properties.

**Test for Tannins:** 1ml of extract was added to 2ml of 5% ferric chloride. Formation of dark blue or greenish black indicates the presence of tannins.

**Test for Saponins**: 2ml of extract was added to 2ml of distilled water with continuous shaking in a graduated cylinder for 15mins length wise. The formation of 1cm layer of form indicated the presence of saponins.

**Test for Alkaloids:** 2ml of concentrated hydrochloric acid was added to 2ml of extract. Few drops of Mayer's reagent were added. Presence of green or white colour precipitate indicates the presence of alkaloids.

**Test for Flavonoids:** 1ml of 2N sodium hydroxide will be added to 2ml of extract. Presence of yellow colour indicates the presence of flavonoids.

**Test for Phenols:** 2ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green colour indicates the presence of phenols.

**Test for Terpenoids:** 0.5ml of the extract was treated with 2ml of chloroform and concentrated sulphuric acid. Formation of red brown colour at the interface indicates the presence of terpenoids.

**Test for Cardiac Glycosides:** 2ml of glacial acetic acid and few drops of ferric chloride was added to 0.5ml of the extract. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

**Test for Resins;** 2ml of extract plus equal volume of acetic anhydride solution, then drops of conc. $H_2SO_4$ gives colophony resins (violet coloration indicates the presence of resins).

**Test for Steroids:** To 1ml of extract equal volume of chloroform was added and a few drops of concentrated sulphuric acid added appearance of brown ring indicates the presence of steroids and appearance of bluish ring will indicates the presence of phytosteroids.

**Biochemical analysis:**

Biochemical analysis was carried out to determine the liver function by determining the serum activities of AST, ALT and ALP, as well as the concentrations of Albumin, conjugated and total Bilirubin. Kidney function parameters which include Urea, Creatinine and electrolytes were also analyzed using Automated Biochemical Analyzer.

**Statistical Analysis**

The data obtained were analyzed by one-way ANOVA in SPSS version 23.0 and expressed as mean ± standard deviations. Statistical significance was determined Duncan's post hoc test to compare the mean between the normal control and test groups. The levels of statistical significance was set at $p<0.05$.

## RESULTS AND DISCUSSION

### Results

**Phytochemical composition of ethanol extract of *P. urinaria***

Table 1 shows both qualitative and quantitative phytochemical composition of *P. urinaria*. The results showed the presence of saponins (8.84 g/100g), tannins (1.32 g/100g), phenols (0.11 g/100g), flavonoids (0.512 g/100g) and alkaloids (0.038 g/100g) while cardiac glycosides, Resins, terpenoids and steroids were not detected.

**Table 1:** Phytochemical composition of ethanol extract of *Phyllantus urinaria*

| Phytochemicals | Qualitative | Quantitative (g/100g) |
|---|---|---|
| Saponins | + | 8.84 |
| Tannins | + | 1.32 |
| Phenols | + | 0.11 |
| Flavonoids | + | 0.512 |
| Alkaloids | + | 0.038 |

| | | |
|---|---|---|
| Cardiac Glycoside | - | ND |
| Resins | - | ND |
| Terpenoids | - | ND |
| Steroids | - | ND |

**Key: (+)** = Present, (-) = Absent. ND = Not detected

**Effect of ethanol extract of *P. urinaria* on Liver function parameters**

The Table 2 below shows the effect of *Phyllantus urinaria* on the liver function parameters in Albino rats. The activity of ALP was significantly ($p < 0.05$) higher in both the 1000mg/kg b.w (232.66±14.02) and 500mg/kg/b.w (462.00±64.45) when compared to the control (213.83±11.67). There was no significant ($p>0.05$) decrease in ALT activity of 1000mg/kg b.w (25.90±5.36) when compared with the control (31.91±6.41) but at 500mg/kg b.w (45.80±11.05), the activity of ALT significant ($p>0.05$) increased when compared with the control (31.91±6.41). There was a significant ($p<0.05$) decrease in AST activity of 1000mg/kg b.w (16.90±11.79) and 500mg/kg b.w (16.13±15.59) when compared with the control (31.83±25.59). Also the Bilirubin concentration showed significant ($p< 0.05$) decrease at 1000mg/kg b.w (2.76±1.67) and no significant ($p>0.05$) increase at 500mg/kg b.w (7.16±2.76) when compared with the control (6.16±6.16). ALB values show no significant increase ($p > 0.05$) at 1000mg/kg b.w (3.40±.88) and 500mg/kg b.w (3.63±.51) when compared with the control (3.16±.59).

Table **2.** Effect of ethanol extract of *P. urinaria* on some Liver function parameters.

| **GROUPS** | ALP (U/L) | ALT (U/L) | AST (U/L) | BIL (μmol/L) | ALB(g/dL) |
|---|---|---|---|---|---|
| CONTROL | 213.83±11.67$^{a}$ | 31.91±6.41$^{a}$ | 31.83±5.59$^{a}$ | 6.16±6.16$^{a}$ | 3.16±0.59$^{a}$ |
| 1000mg/kg/b.w | 232.66±14.02$^{b}$ | 25.90±5.36$^{a}$ | 16.90±11.79$^{b}$ | 2.76±1.67$^{b}$ | 3.40±0.88$^{a}$ |
| 500mg/kg/b.w | 462.00±64.45$^{c}$ | 45.80±11.05$^{b}$ | 16.13±15.59$^{b}$ | 7.16±2.76$^{a}$ | 3.63±0.51$^{a}$ |

Result are presented as Mean ± Standard deviation (n=4). Mean values with different letters as superscripts are considered statistically significant at $p < 0.05$.

**Effect of ethanol extract of *P. urinaria* on Kidney function parameters**

Table 3 shows that there was a non-significant decrease in Serum $HCO_3^-$ concentrations in both 1000mg/kg/b.w (8.00±4.57) and 500mg/kg b.w (9.76±4.46) when compared to the control (11.56±5.64). Also, for serum UREA, there was a non-significant decrease in both 1000mg/kg b.w (15.17±9.85) and 500mg/kg b.w (7.94±6.36) concentration when compared to the control (19.45±8.55). In creatinine, there was a significant increase in both 1000mg/kg b.w (70.56±24.80) and 500mg/kg b.w (155.53±15.01)

concentration when compared to the control ($39.75\pm2.74^{a}$). $Na^{+}$ concentration shows non-significant increase in 1000mg/kg b.w (76.00±23.06) and 500mg/kg b.w (40.33±23.43) when compared to the control (56.16±28.19) group.



**Table 3 ;** Effect of ethanol extract of *P. urinaria* on Kidney function parameters

| **Groups** | $HCO_3^-$(µmol/L) | Urea (µmol/L | Creatinine (µmol/L) | $Na^+$ (µmol/L) |
|---|---|---|---|---|
| CONTROL | 11.56±5.64[a] | 19.45±8.55[a] | 39.75±2.74[a] | 56.16±28.19[a] |
| 1000mg/kg/b.w | 8.00±4.57[a] | 15.17±9.85[a] | 70.56±24.80[b] | 76.00±23.06[b] |
| 500mg/kg/b.w | 9.76±4.46[a] | 7.94±6.36[b] | 155.53±15.01[c] | 40.33±23.43[c] |

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Result are presented as Mean ± Standard deviation (n=4). Mean values with different letters as superscripts are considered statistically significant at $p < 0.05$.

**Discussions.**

This study was focused of determining the phytochemical composition of P. urinaria ethanol etract and determining its effects on the liver and kidney function parameters. The results showed the presence of saponins (8.84 g/100g), tannins (1.32 g/100g), phenols (0.11 g/100g), flavonoids (0.512 g/100g) and alkaloids (0.038 g/100g) while cardiac glycosides, Resins, terpenoids and steroids were not detected. This finding is similar to (Igwe et al., 2007) who stated that phyllantus plant contain high levels of saponins and tannins with low content of cyanogenic glycosides. P. amarus has also been reported to have high amounts of alkaloids and phenols (Komuraiah *et al*., 2009), alkaloids, tannins, and flavonoids (Mazumder et al., 2009), flavonoids, tannins, alkaloids, terpenoids, steroids, saponins and cardiac glycosides (Obianime and Uche, 2009) which are in line with our findings. The presence of these phytochemicals are likely to be behind its pharmacological effects as reported by many authors.

The activity of ALP was significantly ($p < 0.05$) higher in both the 1000mg/kg b.w and 500mg/kg/b.w when compared to the control. There was no significant ($p>0.05$) decrease in ALT activity of 1000mg/kg b.w compared to the control but at 500mg/kg b.w, the activity of ALT significant ($p>0.05$) increased when compared with the control. There was a significant ($p<0.05$) decrease in AST activity of 1000mg/kg b.w (16.90±11.79) and 500mg/kg b.w (16.13±15.59) when compared with the control. Also the Bilirubin concentration showed significant ($p< 0.05$) decrease at 1000mg/kg b.w and no significant ($p>0.05$)

increase at 500mg/kg b.w when compared with the control. ALB values increased non-significantly ($p > 0.05$) at 1000mg/kg b.w and 500mg/kg b.w when compared with the control.

The outcome on the liver function status may be in disagreement with other researchers (Bhattacharjee and Sil, 2006; Sabir and Rocha, Renuka and Rahim, Bhattacharjee, 2007 Harish and Shivanandappa, 2006) who reported that Phyllanthus indicates hepatoprotective effect against acetaminophen-induced toxicity. They also claimed that the aqueous extract of Phyllanthus inhibited paracetamol induced hepatotoxicity in mice. According to them, fishes pretreated with Phyllanthus niruri extract were protected against paracetamol-induced hepatotoxicity when compared to control. They also reported that a protein isolated from Phyllanthus niruri protects against oxidative damage of hepatocytes induced by carbon tetrachloride. Both aqueous and methanol extracts of Phyllanthus niruri have been demonstrated to possess hepatoprotective effect according to the researchers.

Table 3 showed a non-significant decrease in Serum $HCO_3^-$ in both 1000mg/kg/b.w and 500mg/kg b.w concentration when compared to the control. Also, for serum UREA, there was a non-significant decrease at both 1000mg/kg b.w and 500mg/kg b.w concentration when compared to the control. For creatinine, there was a significant increase at both 1000mg/kg b.w and 500mg/kg b.w concentration when compared to the control. $Na^+$ concentration showed a non-significant increase at 1000mg/kg b.w and 500mg/kg b.w when compared to the control group.

According to (Adeneye and Senebo, 2008), aqueous extract of Phyllanthus at doses of 200 mg and 400 mg/kg/day for 14 days, were found to protect against the nephrotoxic effect of paracetamol and gentamicin in rat, by maintaining the level of blood urea nitrogen and serum creatinine within the normal range compared to control group. In another study, the ethanol extract of the leaves of the plant was investigated for its nephroprotective activity against gentamicin induced nephrotoxicity in rats. Co-administration of the extract with gentamicin prevented kidney and improved all nephrotoxic parameters (physical, urinary and blood) observed (Reddy et al., 2017). A study by (Peters et al., 2015) revealed significant decrease in plasma concentrations of creatinine and urea in extract treated groups when compared to negative control value and significant increase in plasma concentrations of $Na^+$ and $HCO3^-$

when compared to negative control value which is a contradiction of the current study at 500mg/kg b.w on sodium ion but agrees with sodium ion at 1000mg/kg b.w. Our study also agrees with (Peters et al., 2015) who found a decrease in bicarbonate ions.

**Conclusion.**

The result of this study suggests that the extracts of *Phyllantus urinaria* at 1000 and 500mg/kg b.w may not have prevented the hepatic and renal tissues challenged by paracetamol because the liver enzymes ALP and ALT as well as Bilirubin and albumin predominantly remained high. While urea concentration reduced, creatinine and sodium ions remained significantly high. Therefore, a histological examination of the organs is recommended to ascertain the effects of the paracetamol and extracts on the tissues.

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