OPEN ACCESS

Orapuh Journal

ORIGINAL ARTICLE

Journal of Oral & Public Health

Phytochemical study and subacute toxicity in vivo evaluation of *Phyllanthus odontadenius* (Phyllanthaceae) aqueous extracts

Nsendo, M. K. O.[1], Kikakedimau, N. R.[2,3], Mbozo, M. A.[1], Taba, K. M.[4], Ngbolua, K. N.[5], & Luyindula, N. S.[2]

[1]*Section of Laboratory Techniques, Higher Institute of Medical Techniques, (ISTM-Kinshasa), Kinshasa, Democratic Republic of the Congo*
[2]*Section of Applied Microbiology, Department of Radiobiology, General Commission for Atomic Energy / Regional Center for Nuclear Studies of Kinshasa (CGEA/CREN-K), Kinshasa, Democratic Republic of the Congo*
[3]*Section of Exact Sciences, Department of Chemistry-Biology, High Institute of Education, Kenge Town, Kwango Province, Democratic Republic of the Congo*
[4]*Laboratory of Chemistry and Industry, Department of Chemistry, Faculty of Sciences, University of Kinshasa, Kinshasa XI, Democratic Republic of the Congo*
[5]*Department of Biology, Faculty of Sciences, University of Kinshasa, Kinshasa XI, Democratic Republic of the Congo*

ARTICLE INFO

Received: *13 February 2025*
Accepted: *09 April 2025*
Published: *22 April 2025*

Keywords:
Chronic in vivo toxicity, phytochemical screening, *Phyllanthus odontadenius*, Wistar rat

Peer-Review: *Externally peer-reviewed*

Correspondence to:
Prof. Dr. Kikakedimau Nakweti Rufin
frankciakika@gmail.com

To cite:
Nsendo, M. K. O., Kikakedimau, N. R., Mbozo, M. A., Taba, K. M., Ngbolua, K. N., & Luyindula, N. S. (2025). Phytochemical study and subacute toxicity in vivo evaluation of *Phyllanthus odontadenius* (Phyllanthaceae) aqueous extracts. *Orapuh Journal, 6*(3), e1229
https://dx.doi.org/10.4314/orapj.v6i3.29

ISSN: *2644-3740*

Published by Orapuh, Inc. (info@orapuh.org)

Editor-in-Chief: Prof. V. E. Adamu
Orapuh, Inc., UMTG PMB 405, Serrekunda, The Gambia, editor@orapuh.org.

ABSTRACT

Introduction
Phyllanthus odontadenius is recognised for its rich composition of natural substances, notably secondary metabolites, which offer promising applications in pharmacology and the food industry.

Purpose
This study sought to identify the major secondary metabolites present in *P. odontadenius* and to assess the in vivo subacute toxicity of its aqueous extracts.

Methods
Aqueous and methanolic extracts of *P. odontadenius* samples collected from Kinshasa, Kongo-Central, and Kwango (Democratic Republic of the Congo) were subjected to phytochemical screening and thin-layer chromatography (TLC). Subacute toxicity of the aqueous extract was evaluated in Wistar albino rats administered 500 mg/kg body weight via oral gavage for 14 consecutive days. Body weights were measured before and after treatment. Post-treatment, the weights of the liver, heart, and kidneys were recorded, and biochemical markers (urea, creatinine, AST, ALT, GGT, and ALP) were analysed.

Results
Phytochemical analysis confirmed the presence of alkaloids, polyphenols, flavonoids, terpenes, anthocyanins, and steroids, while saponins were absent. A notable increase in body weight was observed in all rats. Organ weights varied across extract sources, with the liver from the Kenge group showing the highest weight (4.23 ± 0.04 g). Biochemical profiles showed slight to marked elevations in liver enzymes and kidney markers across treatment groups compared to controls. Particularly, the Kenge extract group recorded the highest AST (322.00 ± 36.68 U/L) and ALP (431.4 ± 32.23 U/L) levels, suggesting possible hepatic stress or damage.

Conclusion
Although *P. odontadenius* is traditionally used in herbal medicine, findings from this study suggest that chronic use of its aqueous extract may pose risks to sensitive organs, especially the liver. Further studies, including histological analysis and isolation of potential toxic constituents, are recommended to support its safe use.

INTRODUCTION

Phyllanthus odontadenius plants have the capacity to produce a wide variety of natural substances, including secondary metabolites that represent an important source of molecules usable in pharmacology and the food industry. Mao et al. (2016) reported several compounds isolated from the *Phyllanthus* genus, while Geethangili and Ding (2018) noted that *P. urinaria* L. is a rich source of lignans, tannins, flavonoids, phenolics, terpenoids, and other secondary metabolites. These plant-based secondary metabolites, produced in very small quantities—over 100,000 molecules have been identified—are chemically classified into isoprene compounds (isoprenoids or terpenoids), phenylpropanoids and heterosides, and alkaloids (Ayoola et al., 2008; Boullard, 2001).

Secondary metabolites serve vital functions for plant survival, including defence against herbivores and pathogens, and promotion of growth and germination. They are also widely used by humans in various fields such as cuisine, agriculture (e.g., herbicides), and medicine (e.g., antibiotics, antiparasitics, antioxidants, drugs, antiarrhythmics, antifungals) (Gerda & Vigan, 2012; Borel et al., 2005; Bidie et al., 2001). Given this biological wealth, medicinal plants—particularly *P. odontadenius*—are employed to treat various diseases such as dysentery, intestinal pain, malaria, and fungal infections. They also exhibit pharmacological activities such as anticancer, hepatoprotective, antidiabetic, antimicrobial, and cardioprotective effects (Geethangili & Ding, 2018).

Studies have demonstrated that *P. odontadenius* has antiviral, antibacterial, antiamoebic, and antifungal properties. Given the medicinal and agri-food importance of this plant, it is essential to investigate whether its aqueous and methanolic extracts from the N'djili Valley in Kinshasa, Kasangulu in Kongo-Central, and Kenge in Kwango Province contain secondary metabolites beneficial to humans, while also assessing the presence of any potentially toxic compounds (Watt, 1992). The aim of this study was to conduct a phytochemical analysis to identify secondary metabolites and to evaluate the chronic in vivo toxicity of *P. odontadenius*. Clinical symptoms induced by toxic substances are often assessed via biochemical parameters, which play a significant diagnostic role (Rosidah et al., 2024).

Evaluating kidney and liver function is vital for profiling the toxicity of plant extracts and ensuring the survival of an organism. Renal and hepatic functions can be assessed using standard biomarkers: renal function via urea and creatinine levels, and liver function via ALT and AST levels (Rosidah et al., 2024). ALT is most specific to the liver, whereas AST and ALP are found in many organs, including cardiac and skeletal muscle, bones, and the placenta. These enzymes may be elevated in the event of tissue damage. A disturbance in transaminase levels may indicate toxic anamnesis due to medications, alcohol, or food supplements (Vande Berg & Stärkel, 2019). Many medications, including plant-based remedies and nutritional supplements, can asymptomatically elevate liver enzymes such as ALT, AST, and ALP, with some leading to drug-induced liver injury (Tholey, 2024).

One clinical use of serum GGT levels is to determine the source of elevated alkaline phosphatase. Since GGT is not elevated in bone disease, concurrent elevation of ALP and GGT suggests a hepatobiliary rather than skeletal source (Lee et al., 2012). GGT is found at the cell membrane level of multiple organs, particularly the kidneys and pancreas, though its blood activity is primarily of hepatic origin. It is located in the plasma membrane of renal tubule cells and bile canaliculi, as well as the endoplasmic reticulum of hepatocytes. The enzyme is of diagnostic interest because it is released into the bloodstream during hepatobiliary diseases. Its measurement can help detect liver diseases and tumours (Murray et al., 2013).

This study aimed to determine the secondary metabolites present in *P. odontadenius* through phytochemical analysis and assess the plant's subacute toxicity via biochemical testing to ensure its safe use. It is a contribution to expanding the knowledge on the potential toxicity of *P. odontadenius*, especially through the evaluation of biochemical parameters (urea, creatinine, liver enzymes) related to vital organs such as the kidneys, heart, and liver. These findings support previous research by Nakweti et al. (2023), who evaluated aqueous extracts (2500 mg/mL) of *P. odontadenius* in NMRI mice.

METHODS

Site and Study Period

This study was conducted at the Radiobiology Department of the General Commission for Atomic Energy/Regional Center for Nuclear Studies of Kinshasa (CGEA/CREN-K) for the preparation of powders from harvested plants. The treatment with *P. odontadenius* extracts, the phytochemical study, and the chronic *in vivo* toxicity assessments were carried out at the Pharmacognosy and Biochemistry Laboratory of the National Institute of Biomedical Research (INRB), Kinshasa/Gombe, Democratic Republic of the Congo (DRC), from October 11, 2019, to April 12, 2023.

Plant Material

Samples of the aerial parts (stems, leaves, and seeds) of *P. odontadenius* were collected in October 2019 from the N'djili River Valley, Kenge, and Kasangulu. The plant material was identified at the Herbarium of the Department of Biology, Faculty of Sciences, University of Kinshasa.

Animal Material

Wistar albino rats from the INRB were used for the subacute toxicity tests of the aqueous extracts of *P. odontadenius*.

Preparation of Plant Extracts

All aerial parts of *P. odontadenius* were used. After drying, the plant material was ground into powder for chemical screening.

Preparation of Aqueous Extracts

Samples of *P. odontadenius* were dried in the Radiobiology Laboratory at CGEA/CREN-K, away from light, humidity, and dust. After approximately ten days, the material was ground using a Thomas Scientific mill. The resulting powder was sieved through a 500 µm mesh. For decoction, 20 g of the powder was weighed using a precision balance and dissolved in 200 ml of distilled water. The mixture was heated in a water bath at 100 °C for 15 minutes. The resulting decoction was filtered using Whatman No. 1 filter paper and collected in a clean bottle. A portion (1.5 ml) of the filtrate was placed in an Eppendorf tube and dried in an oven at 45 °C to obtain a dry extract, which was then used to prepare different dilutions. The remaining filtrate was reserved for chemical screening.

Preparation of Methanolic Extracts

To prepare the methanolic extract, 20 g of *P. odontadenius* powder was mixed with 200 ml of 80% hydromethanolic solution. The mixture was shaken to achieve homogeneity and left at room temperature for 48 hours. It was then filtered using Whatman No. 1 filter paper. A 1.5 ml aliquot of the filtrate was dried in an oven at 45 °C to obtain a dry extract.

Phytochemical Screening

Chemical Screening in Test Tubes

The objective of the chemical screening was to identify phytochemical groups potentially responsible for pharmacological activity. The commonly tested chemical groups included alkaloids, saponins, reducing sugars, polyphenols, catechic and gallic tannins, flavonoids, quinones, and anthocyanins. Screening was conducted on aqueous and methanolic extracts following standard protocols (Harborne, 1998; Mabry et al., 1970, as cited in Nakweti et al., 2012; Kikakedimau et al., 2019).

Thin Layer Chromatography (TLC)

The presence of flavonoids, terpenes, steroids, and alkaloids was confirmed via TLC using silica gel plates 60F254 (10 x 10 cm) (Harborne, 1998; Nakweti et al., 2012).

TLC for Terpenes and Steroids in Chloroform Extracts

On a 60F254 silica plate (10 x 10 cm), 5 µl of each extract and 2 µl of reference substances (oleanolic acid, α-amyrin, sitosterol, or aesin) were applied. The plates were dried and then developed using a chloroform/ethyl acetate (2:3) solvent system. The presence of terpenes and steroids was revealed using anisaldehyde-sulfuric acid reagent. Spots with brownish-blackish coloration indicated positive results. Observations were made in daylight or under UV light at 366 nm. Controls included sitosterol, stigmasterol, α-amyrin, and β-amyrin (1 mg/ml in $CHCl_3$).

TLC for Alkaloids and Flavonoids

The plate was sprayed with Dragendorff's reagent to reveal alkaloids, which appeared as orange-yellow to brown-yellow spots. Controls included quinine, yohimbine, and caffeine (1 mg/ml in MeOH).

For flavonoids, the Neu reagent was used, and observations were made under UV light at 366 nm. Orange-yellow or greenish spots indicated flavonoid presence. Controls included rutin, caffeic acid, chlorogenic acid, quercetin, and hyperoside (1 mg/ml in methanol).

Biological Study

Evaluation of Subacute toxicity

Aqueous extracts of *P. odontadenius* collected from Kinshasa, Kasangulu, and Kenge were used to evaluate subacute toxicity in Wistar albino rats obtained from the INRB animal facility. A total of 15 rats were used in this study.

Experimental Protocol

Rats were marked using picric acid on the head, back, tail, and abdomen for identification. Their body weights were measured on the first and last days of the experiment using a KERN scale.

Preparation of Doses

The process for crude extract preparation was adapted from Nakweti et al. (2024), and the dosing method followed Nakweti et al. (2023). A 500 mg/kg body weight dose was prepared (100 mg/ml), and administered to a 150 g rat. Dosages for other rats (mean weight: 107.63 ± 12.72 g) were calculated based on individual body weights.

Rats were divided into four groups and treated as follows:

- **Batch 1**: Treated with Kenge extract by oral gavage (500 mg/kg bw) for 14 days
- **Batch 2**: Treated with Kasangulu extract by oral gavage (500 mg/kg bw) for 14 days
- **Batch 3**: Treated with Kinshasa extract by oral gavage (500 mg/kg bw) for 14 days
- **Batch 4 (Control)**: Treated with distilled water by oral gavage (10 ml/kg bw) for 14 days

Sacrifice and Blood Collection

On day 15, rats were anaesthetised with ketamine (20 mg/kg bw) and sacrificed. Blood was collected from the heart into dry Eppendorf tubes and centrifuged at 2,500 rpm for 10 minutes. The resulting serum was used to assess biochemical parameters.

Organ Collection

Dissections were performed to harvest the liver, heart, and kidneys for further examination.

Assay of Biochemical Parameters

All biochemical assays were performed using the Cobas C 111 machine, which automatically calculates the concentrations of various biochemical markers.

Statistical Analysis

The results were expressed as mean ± standard error and analysed using Microsoft Excel. Statistical significance was assessed using the Student's *t*-test, with the threshold set at $p < 0.05$ (Rohrmoser, 1986).

RESULTS

Chemical Screening in Tubes

The phytochemical screening of *P. odontadenius* samples collected from three different sites—Kenge, Kasangulu, and Kinshasa—is presented in **Table 1** and **Figures 1 to 3**. According to **Table 1**, all aqueous and methanolic extracts of *P. odontadenius* revealed the presence of gallic and catechic tannins, alkaloids, terpenes, flavonoids, and anthocyanins. However, saponins and quinolines were absent in all the aqueous and methanolic extracts.

Thin Layer Chromatography (TLC)

The phytochemical findings were confirmed using thin layer chromatography (TLC), which revealed the presence of alkaloids, flavonoids, terpenoids, and triterpenoids.

Table 1:
Chemical Compounds Present in ***P. odontadenius*** Extracts

Plant	Extracts	Alk.	Flav.	Ster.	Triterp.	Gal. Tan.	Cat. Tan.	Anth.	Sap.	Quin.
PO1	Aqueous	+	+	+	+	+	+	+	-	±
	Methanolic	+	+	+	+	+	+	+	+	±
PO2	Aqueous	+	+	+	+	+	+	+	-	±
	Methanolic	+	+	+	+	+	+	+	-	±
PO3	Aqueous	+	+	+	+	+	+	+	-	±
	Methanolic	+	+	+	+	+	+	+	+	+

Legend: PO1 = Kinshasa, PO2 = Kasangulu, PO3 = Kenge; + = presence of secondary metabolites, - = absence, ± = trace amounts. Alk. = Alkaloids; Flav. = Flavonoids; Ster. = Steroids; Triterp. = Triterpenoids; Gal. Tan. = Gallic tannins; Cat. Tan. = Catechic tannins; Anth. = Anthocyanins; Sap. = Saponins; Quin. = Quinones.

As shown in **Table 1**, alkaloids, flavonoids, steroids, triterpenoids, tannins, and anthocyanins were consistently present in all extracts. In contrast, saponins and quinones were either absent or detected only in trace amounts.

Growth of Rats in Cages

The mean percentage weight variations in rats, before and after receiving 500 mg/kg of *P. odontadenius* extracts over 14 days, are illustrated in **Figure 1**.

https://dx.doi.org/10.4314/orapj.v6i3.29

It is evident that post-treatment weight gains varied among all groups. The highest mean percentage weight gain (27.39 ± 7.28%) was observed in rats treated with extracts from Kenge, while the lowest (13.75 ± 7.18%) was recorded in rats treated with extracts from Kasangulu. An F-test indicated statistically significant differences ($p < 0.05$) between treatment groups, particularly between the Kasangulu aqueous extract and all other extracts.

Drug Effects on Vital Organs: Heart, Liver, and Kidneys

The effects of aqueous extracts of *P. odontadenius* on the weights of the heart, liver, and kidneys are summarised in Table 2.

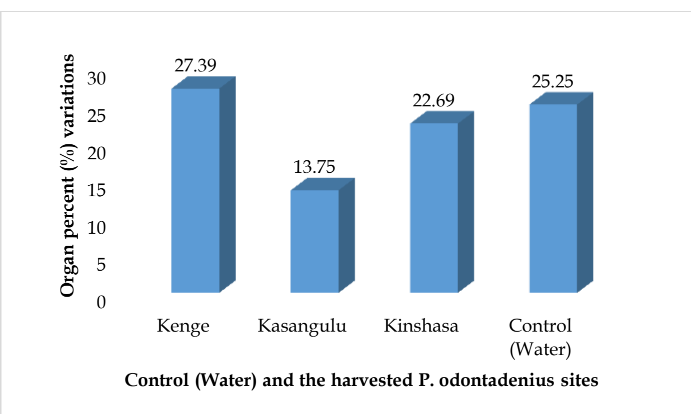
Table 2:
Organ Weight Variations After Treatment with Aqueous ***P. odontadenius*** Extracts

	Lot 1	Lot 2	Lot 3	Lot 4
Heart (g)	0.54 ± 0.03^{a}	0.48 ± 0.07^{ab}	0.45 ± 0.08^{b}	0.45 ± 0.06^{b}
Liver (g)	4.23 ± 0.04^{a}	3.71 ± 1.89^{b}	3.55 ± 0.48^{b}	3.56 ± 0.69^{b}
Left kidney (g)	0.50 ± 0.03^{a}	0.44 ± 0.09^{b}	0.52 ± 0.10^{a}	0.44 ± 0.11^{b}
Right kidney (g)	0.49 ± 0.01^{ab}	0.44 ± 0.10^{b}	0.52 ± 0.10^{a}	0.46 ± 0.12^{b}

Legend: Lot 1 = Kenge extracts; Lot 2 = Kasangulu extracts; Lot 3 = Kinshasa extracts; Lot 4 = Control (distilled water). Superscript letters (^{a}, ^{b}) indicate statistically significant differences at $p < 0.05$.

The highest organ weights were observed in Lot 1 (Kenge), while the lowest were recorded in Lot 4 (control) and Lot 2 (Kasangulu). These differences were statistically significant ($p < 0.05$).

Figure 1:
Mean Percentage Weight Variation in Rats Before and After Treatment



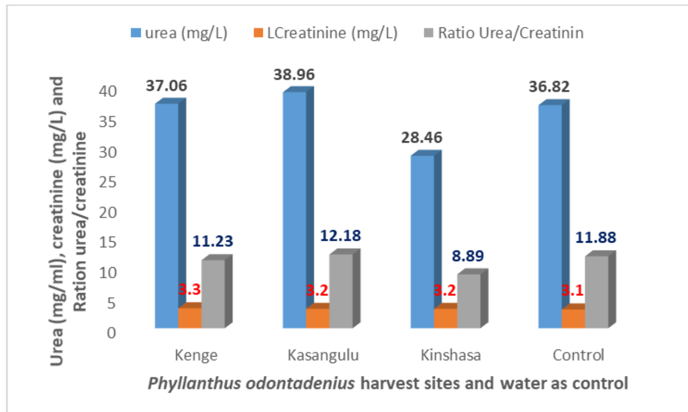
As shown in Figure 1, rats treated with Kenge extracts recorded the highest mean weight gain (27.39 ± 7.28%) compared to the control (25.25 ± 3.51%), Kinshasa (22.69 ± 5.08%), and Kasangulu (13.75 ± 7.18%). Statistically significant differences were found ($p < 0.05$), particularly between the Kasangulu extract group and all other treatment groups.

Biochemical Parameters

a. Renal Function: Urea and Creatinine

High urea levels with normal creatinine levels can suggest causes unrelated to renal dysfunction, such as dehydration, intense exercise, or high protein intake. Creatinine, a muscle metabolism byproduct eliminated by the kidneys, serves as a reliable indicator of renal function (Murray et al., 2013).

Figure 2:
Serum Urea and Creatinine Levels After Treatment



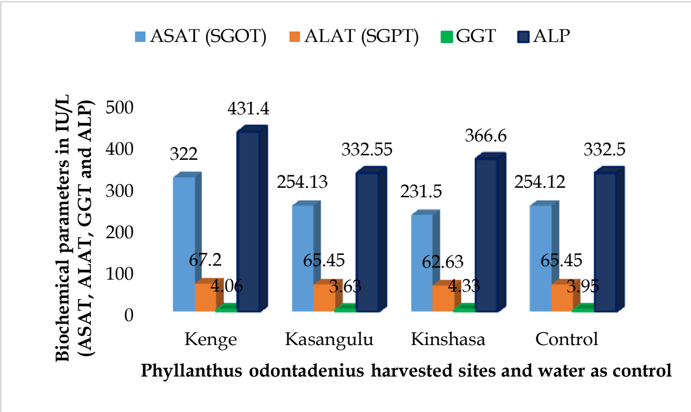
As illustrated in Figure 2, the highest mean serum urea level was observed in rats treated with Kasangulu extracts (38.96 ± 24.64 mg/L), followed by Kenge (37.06 ± 10.30 mg/L), the control (36.82 ± 12.60 mg/L), and Kinshasa (28.46 ± 6.65 mg/L). These differences were statistically significant ($p < 0.05$).

For creatinine, the values were: Kenge (3.3 ± 0.99 mg/L), Kasangulu (3.2 ± 0.86 mg/L), Kinshasa (3.2 ± 1.48 mg/L), and the control (3.1 ± 0.97 mg/L). However, chi-square analysis ($\chi^2 = 8.692$, $p = 0.369$) and F-test ($F_{calculated} = 0.1025 < F_{table} = 4.39$) indicated no statistically significant differences at the 5% threshold.

b. Liver Function: AST, ALT, ALP, and GGT

AST and ALT are markers of hepatocyte injury; ALP indicates biliary obstruction. GGT also supports the assessment of liver function (Murray et al., 2013).

Figure 3:
Serum Levels of Hepatic Enzymes After Treatment



The highest AST level was observed in rats treated with Kenge extracts (322.08 ± 36.68 IU/L), while the lowest was in the control group (254.12 ± 75.57 IU/L). These differences were statistically significant at $p < 0.05$. Elevated AST suggests hepatic cytolysis, i.e., liver cell damage.

ALT, ALP, and GGT levels also varied. Kenge extracts yielded the highest ALP (431.4 ± 32.23 IU/L) and ALT (67.2 ± 7.35 IU/L), while the control had the lowest ALP (332.5 ± 21.25 IU/L). The Kinshasa group recorded the highest GGT value (4.30 ± 0.62 IU/L), while the lowest GGT was seen in the Kasangulu group (3.63 ± 0.80 IU/L).

Statistical analyses showed significant differences ($p < 0.05$) for ALP but no significant differences for ALT and GGT. For GGT, the F-calculated value (0.3870) was less than the F-table value (4.39), indicating no significant difference between treatment groups.

DISCUSSION

The present study aimed to determine the secondary metabolites of *P. odontadenius* through phytochemical analyses and to assess its subacute toxicity via biochemical tests, to evaluate the safety of this plant widely used for the treatment of various diseases.

Phytochemical screening of aqueous and methanolic *P. odontadenius* extracts **(Table 1)** revealed the presence of gallic and catechic tannins, alkaloids, terpenes, flavonoids, anthocyanins, quinones, and steroids. However, saponins and quinones were absent. This absence may be attributed to soil composition and environmental conditions, as reported by Kikakedimau et al. (2013). The current findings corroborate those of Luyindula et al. (2004) and Nakweti et al. (2014), who also found that *P. odontadenius* generally lacks saponins.

The various chromatographic profiles confirmed that the three plant specimens studied belong to the same botanical species. All three samples showed the presence of rutin and quercetin through co-elution of the extracts with reference substances on the TLC plate. Quercetin and rutin appeared in higher concentrations in the Kenge samples compared to the others. This variation is likely due to soil quality and environmental stressors such as UV-B radiation, which can enhance secondary metabolite production (Chaouqi et al., 2023; Khare et al., 2023). Secondary metabolites are natural compounds that mitigate oxidative damage caused by environmental stress, thereby activating plant defence mechanisms (Reshi et al., 2023).

The presence of chlorogenic acid was also detected in all analysed samples. Condensed tannins and derivatives of organic acids, identifiable by blue or green fluorescence, were also observed on the TLC plate. The terpenes and steroid profiles were consistent across samples, and the alkaloid profiles of plants from Kinshasa, Kasangulu, and Kenge were also established. These secondary metabolites have also been reported by Kikakedimau (2018).

After 14 days of treatment with *P. odontadenius* extracts at a dose of 500 mg/kg body weight, the growth patterns of albino Wistar rats varied. Rats treated with aqueous extracts from Kenge showed the highest percentage weight gain (27.39 ± 7.28%) compared to the control group (25.25 ± 0.25%), although the difference was not statistically significant. However, rats treated with extracts from Kasangulu showed a significantly lower weight gain (13.75 ± 7.18%), which was statistically significant at the 5% threshold. Except for the Kenge group, which gained 8.46% more weight than the control, all other treatment groups showed weight reduction: 45.54% for Kasangulu and 10.14% for Kinshasa.

These results align with Bakare et al. (2015), who found that while body weight increased in all rats during treatment, the control group gained more weight than the treated groups. However, our findings do not align with those of Nadra (2023), who observed a significant decrease in body weight in Wistar rats during subchronic treatment with *Ruta montana* methanolic extracts.

The apparent negative effect of *P. odontadenius* aqueous extracts on growth may be due to increased protein and energy metabolism, as proteins, lipids, and carbohydrates are essential for weight gain (Edozien & Switzer, 1978; Rabeh et al., 2019). Growth rate increases with higher dietary protein and is also accelerated by high-fat diets. Rats fed caloric-rich diets showed significant increases in body weight and feed efficiency ratio. Moreover, leptin levels decrease disproportionately with fat loss, reducing satiety and potentially explaining the reduced weight gain (Rosenbaum et al., 2019).

Biochemical parameters including urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were measured to assess the subacute toxicity of *P. odontadenius*, as the liver and kidneys are sensitive to toxic agents and serve as key indicators of pathological changes in both animals and humans (Assi et al., 2016). These liver enzymes are commonly used to screen for liver disease, monitor drug side effects, and evaluate treatment responses (Lee et al., 2012).

Elevated urea and creatinine values typically indicate renal damage, dehydration, or increased protein catabolism. A high urea level with normal creatinine may indicate dehydration, excessive exercise, or a high-protein diet (Murray et al., 2013). The serum urea/creatinine ratio helps differentiate between prerenal, renal, and postrenal (obstructive) uremia, with values >15 considered abnormal.

Our results on urea and creatinine are consistent with Nakweti et al. (2023), who observed a significant difference in urea but not in creatinine at the 5% threshold in NMRI mice. In contrast, the urea-creatinine ratio in our study showed significant differences, ranging from 8.89 ± 0.50 (Kinshasa) to 12.18 ± 0.34 (Kasangulu). These ratios showed significant statistical differences between all samples at the 5% threshold.

Based on the urea and creatinine data (**Figure 2**), it is reasonable to suggest that *P. odontadenius* does not cause direct renal toxicity, as creatinine levels remained within normal limits. Although significant differences were found in urea and urea-creatinine ratios, these did not include differences between treated and control groups.

However, significant differences were observed in AST and ALP values, with increases in all treated groups compared to the control, except for AST in the Kinshasa group. AST levels above 400 IU/L are typically associated with myocardial or skeletal muscle damage (Han et al., 2023), but our AST values did not exceed this threshold, suggesting no severe tissue damage. The elevated AST could instead indicate liver dysfunction.

AST, ALT, and ALP are key indicators of liver function. AST and ALT serve as markers of hepatocyte injury, while ALP is used to detect biliary obstruction (Lee et al., 2012). ALP is produced by various tissues, including the liver and bones, and elevated levels may indicate liver disease, bone disorders, or kidney disease (Murray et al., 2013). In this study, ALP values in treated groups exceeded those in the control group: 29.74% (Kenge), 0.02% (Kasangulu), and 10.26% (Kinshasa). The control group ALP values showed significant differences from those of the Kenge and Kinshasa groups, but not from the Kasangulu group. These findings suggest possible liver damage, which may also explain the AST elevation.

Our biochemical findings are consistent with those of Asare et al. (2011), who studied the subchronic toxicity of *Phyllanthus niruri* and found no significant changes in ALT, GGT, and creatinine, indicating general non-toxicity. However, our AST and ALP results differ, showing significant differences between treated and control rats. In contrast, Nakweti et al. (2023) reported significant differences in ALT and ALP in NMRI mice treated with *P. odontadenius* aqueous extracts.

These discrepancies may stem from species-specific differences and the solvents used. Mild elevations in liver enzymes (less than 2–3 times the upper limit of normal) without symptoms may be considered benign (Lee et al., 2012).

Yeap et al. (2021) also reported increased AST and ALP levels, with 1.23-fold and 1.53-fold elevations, respectively, in mice treated with *Phyllanthus tenellus* aqueous extracts.

No toxicity symptoms were observed, and creatinine levels remained unchanged. Their findings align with Campos de Lima e Silva et al. (2012), who also reported no toxicity at varying doses of *P. tenellus*, though some mice exhibited agitation and stereotyped movements.

The serum urea/creatinine ratio remains a useful diagnostic tool for differentiating between prerenal, renal, and postrenal uremia, with values above 15 considered abnormal.

Some elevated values of biochemical parameters observed in rats treated with *Phyllanthus odontadenius* extracts at a dose of 500 mg/kg body weight for 14 days suggest that such an accumulated dose over a relatively prolonged period can induce organ damage and lead to subacute toxicity (Lee et al., 2012). However, Yan et al. (2022) reported that toxicological studies on *P. fructus* indicated no adverse effects, even at high doses following oral administration.

CONCLUSIONS

In recent years, scientific research has increasingly focused on plant-derived compounds for potential use in the phytopharmaceutical field. Molecules from so-called 'natural' plants are considered important sources of new drugs.

This study aimed to evaluate the subacute toxicity of aqueous extracts of *P. odontadenius*. The biochemical parameters assessed (urea, creatinine, AST, ALT, GGT, and ALP) showed notable variations compared to the control group (administered water), particularly for AST and ALP, which increased from 254.12 ± 75.57 IU/L to 322.08 ± 36.68 IU/L and from 322.5 ± 21.25 IU/L to 431.4 ± 32.23 IU/L, respectively. As these enzymes are indicators of liver dysfunction, their elevation suggests potential damage to sensitive organs, particularly the liver.

These findings, including the increased values of AST and ALP, indicate that the aqueous extracts of *P. odontadenius* may exert harmful effects on vital organs at certain doses, especially the liver. This raises an important question: how can *P. odontadenius*, widely used in the treatment of various conditions (such as malaria and viral infections), still pose hepatotoxic risks? Addressing this critical concern would require further investigation into the specific metabolites responsible for the plant's toxicity, alongside histopathological studies of the affected organs.

Acknowledgement: The authors express their sincere gratitude to the management of INRB for their support in the execution of this research. Special thanks are extended to Professors Désiré Musuyu Munganza and Pyana Pati for providing the treatment solutions and access to animal facilities. Appreciation is also due to the authorities of CGEA/CREN-K for granting additional support during the study.

Ethical Approval: Ethical approval for this study was obtained from the National Institute for Biomedical Research (INRB), which is accredited by the ethics committee recognised by the Ministry of Health and Social Welfare of the Democratic Republic of the Congo (DRC). Consequently, all procedures performed on laboratory animals at the INRB are ethically approved and require no further clearance.

Conflicts of Interest: None declared.

ORCID iDs:

Nsendo, M. K. O.[1]:	Nil identified
Kikakedimau, N. R.[2,3]:	https://oricid.org/0009-0003-0962-7571
Mbozo, M. A.[1]:	Nil identified
Taba, K. M.[4]:	Nil identified
Ngbolua, K. N.[5]:	https://orcid.org/0000-0002-0066-8153
Luyindula, N. S.[2]:	Nil identified

REFERENCES

Asare, G. A., Addo, P., Bugyei, K., Gyan, B., Adjei, S., Otu-Nyarko, L. S., Wiredu, E. K., & Nyarko, A. (2011). Acute toxicity studies of aqueous leaf extract of *Phyllanthus niruri*. *Interdisciplinary Toxicology*, 4(4), 206–210. https://doi.org/10.2478/v10102-011-0031-9

Assi, M. A., Hezmee, M. N., Haron, A. W., Sabri, M. Y., & Rajion, M. A. (2016). The detrimental effects of lead on human and animal health. *Veterinary World*, 9(6), 660–671. https://doi.org/10.14202/vetworld.2016.660-671

Ayoola, G., Coker, H., Adesegun, S., Adepoju-Bello, A., Obaweya, K., Ezennia, E., & Atangbayila, T. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. *Tropical Journal* *of Pharmaceutical Research*, 7(3), 1019–1024. https://doi.org/10.4314/tjpr.v7i3.14686

Bakare, A. A., Adedokun, L. A., Oguntolu, G. O., & Amao, A. A. (2015). In vivo evaluation of genetic and systemic toxicity of aqueous extracts of *Phyllanthus amarus* in mice and rats. *International Journal of Toxicology and Pharmacological Research*, 7(4).

Bidie, A. P., Banga, B., Adou, F., Yapol, J. D. N., & Djamani, A. J. (2011). Activités antioxydante de dix plantes médicinales de la pharmacopée ivoirienne. *Sciences et Nature*, 1(1), 1–11.

Borel, J.-P. (1981). *Comment prescrire et interpréter un examen de biochimie* (4th ed.). Maloine.

Boullard, B. (2001). *Dictionnaire des plantes médicinales du Monde: croyances et réalités*. Estem De Bock.

Campos de Lima e Silva, T., Veras Filho, J., Cavalcanti de Amorim, E. L., Antonia de Souza, I., Paulino de Albuquerque, U., & Cavalcante de Araújo, E. (2012). Acute toxicity study of stone-breaker (*Phyllanthus tenellus* Roxb.). *Revista de Ciências Farmacêuticas Básica e Aplicada*, 33(2), 205–210.

Chaouqi, S., Moratalla-López, N., Alonso, G. L., Lorenzo, C., Zouahri, A., Asserar, N., Haidar, E. M., & Guedira, T. (2023). Effect of soil composition on secondary metabolites of Moroccan saffron (*Crocus sativus* L.). *Plants*, 12(711). https://doi.org/10.3390/plants12040711

Edozien, J. C., & Switzer, B. R. (1978). Influence of diet on growth in the rat. *Journal of Nutrition*, 108(2), 282–290. https://doi.org/10.1093/jn/108.2.282

Geethangili, M., & Ding, S.-T. (2018). A review of the phytochemistry and pharmacology of *Phyllanthus urinaria* L. *Frontiers in Pharmacology*, 9, 1109. https://doi.org/10.3389/fphar.2018.01109

Gerda, & Vigan. (2012). *Progrès en dermato-Allergologie - Besançon Gerda*. John Libbey Eurotext.

Han, J.-H., Kwak, J.-Y., Lee, S.-S., Kim, H.-G., Jeon, H., & Cha, R.-R. (2023). Markedly elevated aspartate aminotransferase from non-hepatic causes. *Journal of Clinical Medicine*, 12(310). https://doi.org/10.3390/jcm12010310

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.

Khare, S., Singh, A., Niharika, Amist, N., Azim, Z., & Singh, N. B. (2023). Secondary metabolites interference on potential of *Solanum lycopersicum* grown under UV-B stress and its impact on developmental attributes of *Capsicum annuum*. *Plant Stress*, 8, 100167. https://doi.org/10.1016/j.stress.2023.100167

Kikakedimau, N. R. (2018). *Increasing secondary metabolites in Phyllanthus odontadenius* M.A. Scholars' Press.

Kikakedimau, N. R., Luyindula, N. S., Doumas, P., Mutambel', H. S. N., Baissac, Y., Cimanga, K. R., Diamuini, N. A., Bulubulu, O. F., & Jay-Allemand, C. (2013). Phytochemical analysis of *Phyllanthus niruri* (Phyllanthaceae) extracts collected in four geographical areas in the Democratic Republic of the Congo. *African Journal of Plant Science*, 7(1), 9–20. https://doi.org/10.5897/AJPS12.087

Kikakedimau, N. R., Musuyu, M. D., Nsendo, M. K. O., Doumas, P., Kahambu, M. S., Taba, K. M., & Luyindula, N. S. (2019). Correlation between antioxidants and antiradical activities with in vitro of *Phyllanthus odontadenius*. *Acta Scientific Medical Sciences*, *3* (7), 144 – 154.

Lee, T. H., Kim, W. R., & Poterucha, J. J. (2012). Evaluation of elevated liver enzymes. *Clinics in Liver Disease, 16*(2), 183–198. https://doi.org/10.1016/j.cld.2012.03.006

Luyindula, N., Tona, L., Lukembila, S., Tsakala, M., Mesia, K., Musuamba, C. T., et al. (2004). In vitro antiplasmodial activity of callus extracts from fresh apical stems of *Phyllantus niruri* L (Euphorbiaceae), Part 1. *Pharmaceutical Biology, 42*(7), 1–7. https://doi.org/10.9734/JPRI/2018/37978

Mao, X., Wu, L.-F., Guo, H.-L., Chen, W.-J., Cui, Y.-P., Qi, Q. S., Li, Q. S., Liang, W.-Y., Yang, G.-H., Shao, Y.-Y., Zhu, D., She, G.-M., You, Y., & Zhang, L.-Z. (2016). The genus *Phyllanthus*: An ethnopharmacological, phytochemical, and pharmacological review. *Evidence-Based Complementary and Alternative Medicine, 2016*, 1–36. https://doi.org/10.1155/2016/7584952

Murray, R. K., Bender, D. A., Botham, K. M., Kennelly, P. J., Rodwell, V. W., & Weil, P. A. (2013). *Harper's Biochemistry* (5th ed.; Translation of the 29th

American Edition by Lionel Domenjoud). De Boeck Supérieur.

Nadra, G. (2023). Étude de la toxicité aiguë et sub-chronique des extraits de *Ruta montana* L. sur les rats albinos Wistar : Effet sur le foie, le rein, l'ovogenèse et l'embryogenèse (Doctoral thesis). http://dspace.univ-setif.dz:8888/jspui/handle/123456789/4096

Nakweti, K. R., Doumas, P., Mutambel', H., Diamuini, N. A., Otono, F. B., Akolomia, J. K., Ndiku, S. L., & Kanyanga, R. C. (2012). Antiplasmodial activity and phytochemical analysis of *Phyllanthus niruri* L. (Phyllanthaceae) and *Morinda lucida* Benth (Rubiaceae) extracts. *Journal of Agricultural Science and Technology A, 2*, 373–383.

Nakweti, R. K., Mwangu-Kabi, O. N., Muganza, D. M., Cimanga, K. R., & Ndiku, S. L. (2023). Acute toxicity and *in vivo* antioxidant activity evaluation of aqueous extracts from *Phyllanthus odontadenius* Müll. Arg. *Journal of Pharmaceutical Research International, 35*(5), 20–33. https://doi.org/10.9734/JPRI/2023/v35i57326

Nakweti, R. K., Ndiku, S. L., Sinou, V., Luyeye, F. L., Mbemba, T. F., Mutambel', D. H., Kanianga, R. C., & Ndofunsu, A. D. (2014). Effects of gamma irradiation on seeds germination, plantlets growth and *in vitro* antimalarial activities of *Phyllanthus odontadenius* Müll A. *American Journal of Experimental Agriculture, 4*(11), 1435–1457.

Rabeh, N. M., El-Masry, H. G., Ghallab, E. M., Elghandour, H. M., & Hassanien, A. M. (2019). Evaluation of efficacy diet supplemented with some local formulas on body weight and haematological parameters of rats. *Egyptian Journal of Nutrition and Health, 14*(1), 27–40.

Reshi, Z. A., Ahmad, W., Lukatkin, A. S., & Javed, S. B. (2023). From nature to lab: A review of secondary metabolite biosynthetic pathways, environmental influences, and *in vitro* approaches. *Metabolites, 13*(8), 895. https://doi.org/10.3390/metabo13080895

Rohrmoser, K. (1986). *Manual on field trials under the Cooperation Technique*. CTA & GTZ. *(In French)*

Rosenbaum, J. L., Frayo, R. S., Melhorn, S. J., Cummings, D. E., & Schur, E. A. (2019). Effects of multiple cycles of weight loss and regain on the body weight regulatory system in rats. *American Journal of Physiology-Endocrinology and Metabolism, 317*, E863–E870. https://doi.org/10.1152/ajpendo.00110.2019

Rosidah, I., Renggani, T. N., Firdausi, N., Ningsih, S., Yunianto, P., Permatasari, D., et al. (2024). Acute and subchronic toxicological study of the cocktail extract from *Curcuma xanthorrhiza* Roxb, *Phyllanthus niruri* L., and *Morinda citrifolia* L. *Journal of Toxicology, 2024*, 1–16. https://doi.org/10.1155/2024/9445226

Tholey, D. (2024). Lésions hépatiques provoquées par les médicaments. *Le MANUEL MSD, version pour professionnel de santé*. https://www.msdmanuals.com/fr/professional/troubles-hépatiques-et-biliaires *(Consulted 3/10/2025)*

Vande Berg, P., & Stärkel, P. (2019). Comment interpréter et bilanter une perturbation des transaminases ? *ECU - Congrès de Médecine Générale*, 1–9.

Watt, J. (1992). *Les plantes médicinales et toxiques de l'Afrique australe et de l'Est* (2nd ed.). Londres.

Yan, X., Li, Q., Jing, L., Wu, S., Duan, W., Chen, Y., Chen, D., & Pan, X. (2022). Current advances on the phytochemical composition, pharmacologic effects, toxicology, and product development of *Phyllanthi Fructus*. *Frontiers in Pharmacology, 13*, Article 1017268. https://doi.org/10.3389/fphar.2022.1017268

Yeap, S. K., Yong, C. Y., Faruq, U., Ong, H. K., Mohamed Amin, Z. B., Ho, W. Y., Sharifudin, S., & Jaganath, I. B. (2021). *In vivo* toxicity and antioxidant of pressurised hot water *Phyllanthus tenellus* Roxb. extracts. *BMC Complementary Medicine and Therapies, 21*(86), 1–8. https://doi.org/10.1186/s12906-021-03260-y