

THE SYNERGISTIC EFFECTS OF THE LEAVES OF *MANGIFERA INDICA* (MANGO) AND *FLEURYA AESTUANS* (LAPORTEA), AND ROOTS OF *CYNODON DACTYLON* ON ETHYLENE GLYCOL-INDUCED KIDNEY STONES IN WISTAR RATS

Adamu Musa Abdulkarim*, Gabriel Ajibade, Karderam Bukar Dikwa, Foruh Joseph Appah

Department of Biological Sciences, Faculty of Science, Nigerian Defence Academy, Kaduna State, Nigeria

*Corresponding Author Email Address: abdulkarim4real1@gmail.com

ABSTRACT

Kidney stones are a painful, recurrent condition often treated surgically; plant-based therapies remain underinvestigated. The objective of this study is to evaluate the nephroprotective and anti-urolithic effects of ethanolic extracts of *Mangifera indica* leaves (MIL), *Cynodon dactylon* roots (CDR) and *Fleurya aestuans* leaves (FAL), individually and combined, in an ethylene glycol (EG)-induced rat model. Twenty-four male Wistar rats (110–150 g) were divided into six groups (n = 4): Group 1, control (water); Group 2, EG (0.75% w/v) negative control; Group 3, EG + MIL; Group 4, EG + CDR; Group 5, EG + FAL; Group 6, EG + MIL+CDR+FAL (combined). Extracts were administered orally daily for 28 days concurrently with EG. Body weights were recorded weekly. Serum urea, creatinine, uric acid and calcium were measured; kidney histopathology was performed. Acute toxicity (OECD 423) at 2000 mg/kg showed no mortality. EG induced significant renal impairment: urea 67.62 ± 3.20 mg/dL and creatinine 0.37 ± 0.02 mg/dL (Group 2). Treatment groups showed significant improvements ($p < 0.05$). The combined group restored urea to 27.56 ± 1.21 mg/dL and creatinine to 0.18 ± 0.01 mg/dL (comparable to control). The EG group exhibited tubular dilatation, necrosis and intratubular crystals; treated groups showed reduced lesions, with the combined group showing near-normal kidney architecture. The extract of *Mangifera indica* and *Fleurya aestuans*, as well as the roots of *Cynodon dactylon*, exert nephroprotective and anti-urolithic effects in EG-induced rats, with evidence of synergism when combined.

Keywords: Kidney stones; ethylene glycol; *Mangifera indica*, *Cynodon dactylon*, *Fleurya aestuans*, creatinine, histopathology.

INTRODUCTION

Kidney stones are a painful disorder of the urinary tract. Kidney stones affect about 12% of the world population at some stage in their lifetime, irrespective of age, sex and race, but they occur more frequently in men than in women within the age of 20 to 49 years (Tilahun *et al.*, 2021). The pathogenesis of kidney stones remains incompletely understood, and their treatment remains a challenge. The pain of having a stone has been compared to that of childbirth. The stones grow slowly over several months or years and are made of hard deposits of various minerals, including calcium, uric acid, and oxalate (Prasobh & Revikumar, 2011). Kidneys must maintain an adequate amount of water in the body to remove waste products. If dehydration occurs, high levels of substances that do not dissolve completely (e.g., calcium, oxalate, uric acid) may form crystals that slowly build up into kidney stones (Prasobh &

Revikumar, 2011). Some endogenous factors, like improper metabolism of calcium, oxalic acid, phosphorus, uric acid and nitrogenous waste products; exogenous factors, like urea; also contribute to renal stone formation. Besides, people's food habits, dehydration, hot climate and hard water usage are involved in kidney stone formation (Aishwarya *et al.*, 2021). The etiology of this disorder is multifactorial and is related to genetics and diet. Calcium-containing stones are the most common kidney stones (75–90%), followed by magnesium ammonium phosphate (struvite) (10–15%), uric acid (3–10%), and cystine (0.5–1%) (Mina *et al.*, 2018).

Kidney stones typically leave the body by passage in the urinary stream, and many stones are formed and passed without causing symptoms. If stones grow to sufficient size before passage, on the order of at least 2 to 3 millimeters, they can cause obstruction of the ureter. The resulting obstruction causes dilation or stretching of the upper ureter and renal pelvis as well as muscle spasm of the ureter, trying to move the stone. This leads to pain, most commonly felt in the flank, lower abdomen and groin. Renal colic can be associated with nausea and vomiting. There can be blood in the urine, visible with the naked eye or under the microscope (Prasobh & Revikumar, 2011). The stones may cause various symptoms, including pain, obstruction, infection, and hemorrhage, through the passage of stones in the urinary tract system (Mina *et al.*, 2018). Treatment of renal stones depends on stone size and location. Many therapies, such as diuretics, probiotics, citrate, and chelating agents, are used. However, they have their own pharmacological limitations and side effects with long-term use, and they are not effective in completely removing the stone. So, in the majority of cases, renal stones are removed by surgical treatment like ESWL, ureteroscopy and percutaneous nephrolithotomy, but unfortunately, recurrence of stones was observed in about 50% of cases. However, surgical treatment causes side effects such as hypertension, tubular necrosis, haemorrhage and fibrosis of the kidney (Aishwarya *et al.*, 2021).

Based on ethnobotanical use, this study tested ethanolic extracts of *Mangifera indica*, *Cynodon dactylon* and *Fleurya aestuans* individually and combined, to evaluate their ability to treat EG-induced kidney stones.

MATERIALS AND METHODS

Collection, Identification and Processing of Plant Samples

Leaves/roots were collected from the NDA botanical garden and authenticated at the Department of Biological Sciences, Nigerian

Defence Academy (vouchers were: *Mangifera indica* leaves—NDA/BIOH202509; *Cynodon dactylon* roots — NDA/BIOH202508; *Fleurya aestuans* leaves — NDA/BIOH202510).

Extraction

About 500 g of each shade-dried, powdered plant material was macerated in 95% ethanol (room temperature) for 48 h; extraction was repeated twice for 24 h. Filtrates were pooled and concentrated at 40°C to yield dried extracts, stored at 4°C until use

Qualitative Phytochemical Screening

Alkaloids

Dragendorff's reagent was used, and the method described by Harborne (1998) was adopted. A ground and powdered sample (0.2g) was extracted with 95% ethanol in a Soxhlet extractor for six hours, and the ethanolic extract was evaporated to dryness using a vacuum evaporator at 45°C. The residue was redissolved in 5ml of 1% HCl, and 5 drops of Dragendorff's reagent were added. Colour changes were observed to draw inferences.

Saponin

The persistent frothing test for saponin described by Odebiyi and Sofowora was used. To 1g of each powdered plant sample, 30 ml of tap water was added. The mixture was vigorously shaken and heated. The sample was observed for the formation of froth to draw inference.

Tannins

The method of (Trease & Evans, 2009) was adopted. 0.5 g of powdered each plant sample was dissolved in 5 ml of distilled water, then boiled gently and cooled. 1 ml of this solution was put in a test tube, and 3 drops of ferric chloride solution were added. The colour of the sample was observed to draw inferences.

Terpenoid

The Salkowski test was used; 5 ml of the powder of each plant sample was mixed in 2 ml of chloroform, and 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. Colour changes were observed to draw inferences.

Steroids

2 ml of acetic anhydride was added to 0.5 g of powdered Plant of each sample, followed by 2 ml of sulphuric acid. Colour changes were observed to draw inferences.

Glycoside

The Keller-Killiani test was used; 5 ml of the powdered plant of each sample was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was followed by the addition of 1 ml of concentrated sulphuric acid. Colour changes were observed to draw inferences.

Flavonoids

5 ml of diluted ammonia solution was added to a portion of the aqueous filtrate of each plant extract, followed by the addition of concentrated sulphuric acid. Colour changes were observed to draw inferences.

Quantitative Phytochemical Analysis

Determination of total alkaloid

1 g of each plant sample was macerated with 20 ml of ethanol and 20% sulfuric acid (H_2SO_4) (1:1 v/v). 1 ml of the filtrate was added to 5 ml of 60% H_2SO_4 . After 5 min, 5 ml of 0.5% formaldehyde in 60% H_2SO_4 was mixed with the mixture and allowed to stand for 3 hrs. The absorbance was read at 565 nm using a spectrophotometer.

Determination of total terpenoids

1 g of each plant sample was macerated with 50 ml of ethanol and filtered. To the filtrate, 2.5 ml of 5% aqueous phosphomolybdic acid solution was added, and 2.5 ml of concentrated H_2SO_4 was added and mixed. The mixture was left to stand for 30 min and then made 89 to 12.5 mL with ethanol. The absorbance was taken at 700 nm.

Determination of total flavonoids

The total flavonoid content in the sample was estimated by the method of Chang. A volume of 0.25 ml of each plant sample was diluted to 1.25 ml with distilled water. Sodium nitrite was added, and after six minutes, 0.15 ml of aluminium chloride solution was added. 0.5ml of 0.1 M NaOH was added after 5 min and made up to 2.5 ml with distilled water. 75µl of 5% sodium nitrate was added, and after six minutes, 0.15 ml of aluminium chloride solution was added. 0.5 ml of 0.1 M NaOH was added after 5 min and made up to 2.5 ml with distilled water. The solution was mixed well, and the absorbance was read at 510 nm along with standard quercetin at 5 - 25 µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent/gm of dried sample.

Determination of total saponins

1g of each plant sample was macerated with 10 ml of petroleum ether and decanted into a beaker. Another 10 ml of petroleum ether was added into the beaker, and the filtrate was evaporated to dryness. The residue was then dissolved in 6 ml of ethanol. The solution (2 ml) was put into a test tube with 2 ml of chromogen solution added into it and left to stand for 30 min. The absorbance was read at 550 nm.

Determination of total phenols

1g of each plant sample was macerated with 20 ml of 80% ethanol and filtered. The filtrate (5 ml) was added to 0.5 ml of Folin-Ciocalteu's reagent and allowed to stand for 30 min. Then, 2 ml of 20% sodium carbonate was added, and absorbance was measured at 650 nm.

Determination of total tannins

About 500 mg of each plant sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hr in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCL and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Van-Burden & Robinson, 1981).

Determination of total glycoside

Eight (8) mL of each plant sample was transferred to a 100 mL volumetric flask, and 60 mL of H_2O and 8 mL of 12.5% lead acetate were added, mixed and filtered. 50 mL of the filtrate was transferred into another 100 mL flask, and 8 mL of 47% Na_2HPO_4 was added to precipitate excess Pb^{2+} ions. This was mixed and completed to a volume with water. The mixture was filtered twice through the

same filter paper to remove excess lead phosphate. 10 mL of purified filtrate was transferred into a clean Erlenmeyer flask and treated with 10 mL Baljet reagent. A blank titration was carried out using 10 mL distilled water and 10 mL Baljet reagent. This was allowed to stand for one hour for complete color development. The color intensity was measured colorimetrically at 495 nm.

GC-MS ANALYSIS

GC-MS analysis of extracts was performed to identify volatile and semi-volatile constituents. Peaks were identified by library match (NIST). Reported values are % area.

Preparation of *Mangifera Indica* leaves (MIL), *Cynodon dactylon* Roots (CDR) and *Fleurya aestuan* Leaves (FAL) Extracts.

The leaves of *Mangifera indica*, *Cynodon dactylon* roots and *Fleurya aestuan* leaves were collected, cleaned, shade-dried and milled into powder using a mortar and pestle. About 500 g was macerated in 95% ethanol at room temperature for 48 hours. The entire process was repeated twice for 24 hours each. The ethanol extract was pooled together and filtered using filter paper (Whatman size No. 1). The filtrate was concentrated on a water bath at 40 °C. The dried extract obtained was weighed and kept in an airtight bottle in a refrigerator at 4 °C until required for use.

Experimental Animals

A total of 24 male Wistar rats aged 8–10 weeks and weighing 110–150 g were purchased from the Laboratory Animal Centre of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, where the laboratory work was carried out. The rats were allowed two weeks of acclimatization before being used for experimentation. The animals were housed in standard metal cages, maintained under standard laboratory conditions and fed with standard rat pellets and potable water ad libitum.

Acute Oral Toxicity Study (LD₅₀)

The safe dose of ethanol of *Mangifera indica* leaves (MIL), *Cynodon Dactylon* root (CDR) and *Fleurya aestuan* leaves (FAL) extract was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines 423 (adopted in 2001). 15 male Wistar rats were used in the study on the acute toxicity of *Mangifera indica* leaves (MIL), *Cynodon dactylon* root (CDR) and *Fleurya aestuan* leaves (FAL) extracts. After a 12-hour fast, a limited dose of 2000mg/kg was administered orally to groups of 3 animals for each dose. At 0, 30, 60, 120, 180 and 240 min, signs and symptoms of toxicity and mortality were observed for the *Mangifera indica* leaves (MIL), *Cynodon dactylon* root (CDR) and *Fleurya aestuan* leaves (FAL) extracts. Thereafter, for 14 days, toxicity was delayed. For 14 days, the animals were regularly monitored for any signs of mortality, including general motor activity, convulsions, writhing and drowsiness. After the observation period, the number of survivors was reported. (Venkatesan *et al.*, 2023).

Induction Of Kidney Stones In Wistar Rats / Ethylene Glycol-Induced Nephrolithiasis In Rats

24 Male Wistar rats were tested for anti-nephrolithiatic activity using a model of EG-induced nephrolithiasis, where activity was absent; they were given EG (0.75% w/v) orally for 28 days to induce nephrolithiasis (Venkatesan *et al.*, 2023).

Experimental Set Up

Kidney stones were induced in the Wistar rats by giving them 0.75% ethylene glycol by oral gavage for 28 days. Calcium, uric acid, urea and creatinine were determined as biomarkers in addition to kidney histology and oxidative stress markers.

Experimental Animals Grouping

Experimental animals (Wistar rats) were grouped into 6 groups of 4 each as follows;

Group 1: (positive control group) was administered only water,

Group 2: (negative control group) was administered only ethylene glycol (0.75%w/v)

Group 3 (EG-treated) was administered only *Mangifera indica* (MIL) extract once daily for 28 days.

Group 4 (EG-treated) received only *Cynodon dactylon* root (CDR) extract once daily for 28 days.

Group 5 (EG-treated) received only *Fleurya aestuan* leaves (FAL) extract once daily for 28 days.

Group 6: was administered both *Magifera indica* (MIL) extract, *Cynodon dactylon* roots extract (CDR), and *Fleurya aestuan* leaves (FAL) extract once daily.

Collection of Serum

Under a light ether anaesthetic, glass capillaries were used to draw blood from the rat's retro-orbital plexus. A total of 2 mL Eppendorf tubes were used to collect the blood. It was centrifuged at 5000 rpm for 20 min to separate the serum after allowing it to clot in the open for 15 min. The collected serum was kept at 20 °C to estimate additional biochemical parameters such as calcium, creatinine, uric acid and urea. (Venkatesan *et al.*, 2023).

Histopathological Studies

Histological examinations were carried out for kidney tissues of the experimental rats. All rats were sacrificed in a humane manner using Isoflurane anesthesia at the end of the 28th day. One kidney from each group of animals was removed during the sacrifice process, preserved in 10% formalin after being washed with normal saline and then histological parameters were examined. The tissue was cleaned, alcohol was used to dehydrate it, xylene was used to clarify it, and paraffin blocks were created. Using a rotary microtome, 5 m thick serial slices were made. The sections were deparaffinized in xylene and then hydrated in decreasing alcohol grades. The slides were then exposed to hematoxylin for 10 min before being thoroughly washed with water. These were examined, counterstained with eosin, rinsed with water, dried with escalating levels of alcohol, cleaned with xylene and mounted after drying.

Statistical Analysis

The results were presented as a mean and standard error of the mean. One-way ANOVA was used to statistically examine the differences between the data with the aid of Statistical Package for Social Sciences (SPSS) software to establish the degree of significance at p-values <0.05.

RESULTS

Acute toxicity

No mortality or gross signs of toxicity were observed at 2000 mg/kg during 14 days of monitoring.

Results of qualitative phytochemical constituents of *Mangifera indica* leaves, *Cynodon dactylon* roots and *Fleurya aestuan* leaves.

Table 1: Qualitative phytochemical constituents of *Mangifera indica* leaves, *Cynodon dactylon* roots and *Fleurya aestuan* leaves

Phytochemicals constituents	<i>Mangifera indica</i> leaves	<i>Cynodon dactylon</i> root	<i>Fleurya aestuan</i> leaves
Alkaloid	+	-	-
Flavonoid	+	+	+
Tannins	+	+	+
glycoside	+	+	+
Saponin	-	+	+
Phenols	+	+	+
Terpenoids	+	+	+

The result of the qualitative phytochemical screen of *Mangifera indica* leaves revealed the presence of flavonoids, alkaloids, tannins, phenols, terpenoids and glycosides, but the absence of saponins. The result of *Cynodon dactylon* root showed the

presence of flavonoids, tannins, phenols, terpenoids, saponins and glycosides, but the absence of alkaloids, whereas in *Fleurya aestuan* leaves, flavonoids, tannins, phenols, terpenoids, saponins and glycosides were present, but alkaloids were absent.

Results of Quantitative phytochemical constituents Analysis of *Mangifera indica* leaves, *Cynodon dactylon* roots and *Fleurya aestuan* leaves.

Table 2. Quantitative phytochemical constituents of *Mangifera indica* leaves, *Cynodon dactylon* roots and *Fleurya aestuan* leaves

Phytochemicals constituents	<i>Mangifera indica</i> leaves (%)	<i>Cynodon dactylon</i> root (%)	<i>Fleurya aestuan</i> (%)
Alkaloid	1.18±0.05	0.86±0.03	0.68±0.04
Flavonoid	9.33±0.42	6.92±0.31	8.14±0.28
Tannins	5.65±0.25	2.51±0.15	4.71±0.18
Glycoside	1.59±0.09	1.46±0.05	1.47 ±1.06
Saponin	0.99±0.08	1.15±0.06	1.06±0.07
Phenols	6.29±0.25	7.36±0.35	5.67±0.22
Steroids	1.91±0.12	3.01±0.10	2.06±0.11

Data are presented as Mean ± SEM.

There is a highly significant difference at $P < 0.05$ in the concentration of flavonoids, phenols and tannin across the three samples. *Mangifera indica* leaf extract yielded the highest concentration of flavonoids (9.33±0.42) and tannin (5.65±0.25). In contrast, *Cynodon dactylon* root extract recorded the highest

phenolic content (7.36±0.35) and steroid levels (3.01±0.10). Using Dunnett's multiple comparison test, the variation in alkaloid, flavonoid and tannin was statistically significant ($P < 0.05$), while differences in saponin and glycosides were not statistically significant.

Effects of formulation on body weight on 0 day and 28 days on ethylene glycol

Table 3. Effects of treatment formulation on body weight in ethylene glycol-induced kidney stones in Wistar rats (g)

Groups	0 days	7 days	14 days	21 days	28 days
Control	86.33 ± 4.10	93.66 ± 3.50	97.40 ± 4.20	106.33 ± 5.10	111.29± 4.80
Ethylene glycol induced.	103.43± 5.20	126.94± 61.0	132.48± 5.80	140.48± 6.30	137.74± 5.90
<i>Mangifera indica</i> (MIL) extract.	90.63 ± 3.80	115.13± 4.40	117.46± 4.90	125.26±5.20	128.76± 4.70
<i>Cynodon dactylon</i> roots (CDR) extract.	83.63± 4.50	97.24± 3.90	100.23±4.10	111.54± 4.80	120.37±5.20
<i>Fleurya aestuan</i> (FAL) extract.	94.85± 4.70	114.99± 5.00	126.83± 5.50	134.56±6.10	140.37± 6.40
Mixed plans extracts	97.64± 4.90	115.77±5.20	118.91± 5.10	122.74± 5.50	126.13± 5.80

Data are presented as Mean ± SEM.

The negative control group showed a weight decrease from 140.48 ± 6.30 on day 21 to 137.74 ± 5.90 on day 28. In contrast, rats treated with *Mangifera indica* leaves (group 3), *Cynodon dactylon* extracts (group 4) and the combined group formulation (group 6) showed a significantly different growth profile at $P < 0.05$ compared

to the negative control group. Rats treated with *Fleurya aestuan* leaf extracts (group 5) ended with a high weight, but the difference from the negative control's final weight was not statistically significant ($P > 0.05$).

Effect of formulation on urea, creatinine, uric acid and calcium in ethylene glycol

Table 4. Effect of treatment formulation on blood parameters in ethylene glycol-induced kidney stones in Wistar rats (mg/dL)

Groups	Urea	Uric Acid	Creatinine	Calcium
Control	27.14 ± 1.15	9.08 ± 0.41	0.19 ± 0.01	9.94 ± 0.32
Ethylene glycol induced	67.62 ± 3.20	13.22 ± 0.61	0.37 ± 0.02	10.94 ± 0.45
<i>Mangifera indica</i> (MIL) extract.	34.95 ± 1.55	11.22 ± 0.50	0.28 ± 0.01	9.97 ± 0.38
<i>Cynodon dactylon</i> roots (CDR) extract.	30.09 ± 1.40	11.77 ± 0.55	0.20 ± 0.01	10.09 ± 0.41
<i>Fleurya aestuan</i> leaves (FAL) extract.	29.06 ± 1.32	10.22 ± 0.48	0.29 ± 0.01	10.30 ± 0.43
Mixed plant extracts	27.56 ± 1.21	11.78 ± 0.58	0.18 ± 0.01	10.30 ± 0.43

Data are presented as Mean $\pm$ SEM.

The negative control group treated with ethylene glycol resulted in a massive increase in serum urea (67.62 ± 3.20 mg/dL) and creatinine (0.37 ± 0.02 mg/dL) compared to the normal control (group 1). However, groups treated with plant extracts showed a significant reduction in all four biochemical parameters (urea, uric acid, creatinine and calcium); particularly the synergistic group (group 6) showed the most significant effect, reducing urea to 27.56 ± 1.21 and creatinine to 0.18 ± 0.01 , which are statistically comparable to the positive control group. Calcium was also significantly reduced in the *Mangifera indica*- and *Cynodon dactylon*-treated groups at $P < 0.05$ compared to the negative control group.

HISTOPATHOLOGY

4.4 RESULT OF HISTOPATHOLOGY STUDIES

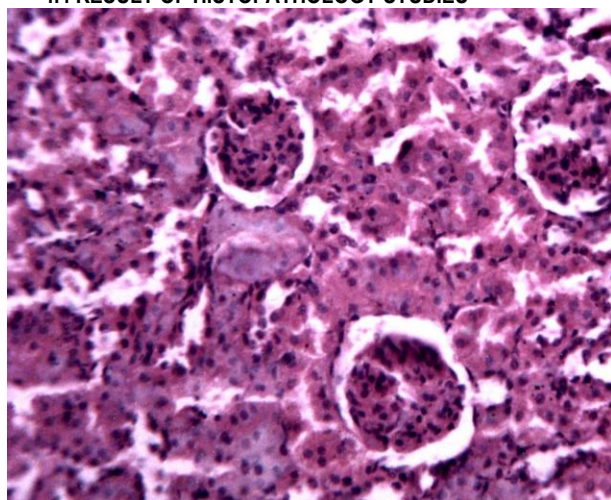


PLATE I: Photomicrograph of the kidney (Grp 1: control; given water only). Note the normal/regular renal architecture showing intact glomeruli and renal tubules. H & E Stain. x250.

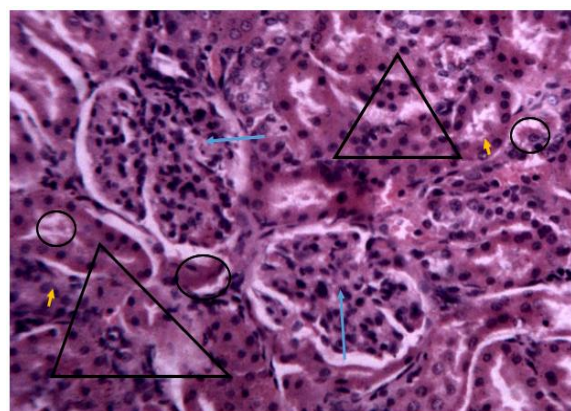


PLATE II: Photomicrograph of the kidney of a Wistar rat with a kidney stone (Grp 2: ethylene glycol, only). Note the generalized distortion/irregular outline of the renal parenchyma. Compression or shrinkage of the glomeruli (arrows), dilatations of renal tubules (arrow heads), intratubular crystals/deposits (circles) and necrosis (Triangle). H&E stain. x250.

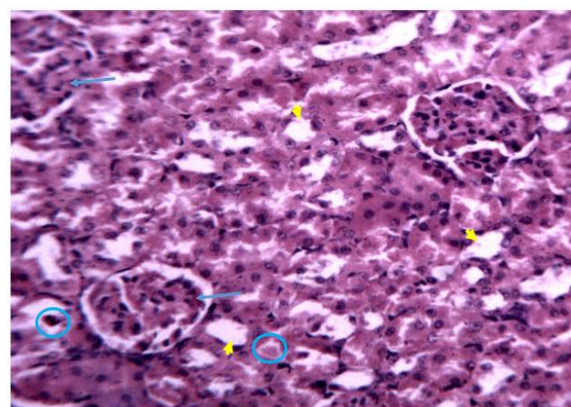


PLATE III: Photomicrograph of the kidney of a Wistar rat with a kidney stone (Grp 3: *Mangifera indica* (MIL)). Note near-normal glomeruli (arrows), moderately dilated renal tubules with vacuolations (arrow heads), some crystals/deposits (circles) and necrosis. H & E Stain. x250.

The Synergistic Effects of the Leaves of *Mangifera Indica* (Mango) and *Fleurya Aestuans* (Laportea), and Roots of *Cynodon Dactylon* on Ethylene Glycol-Induced Kidney Stones in Wistar Rats

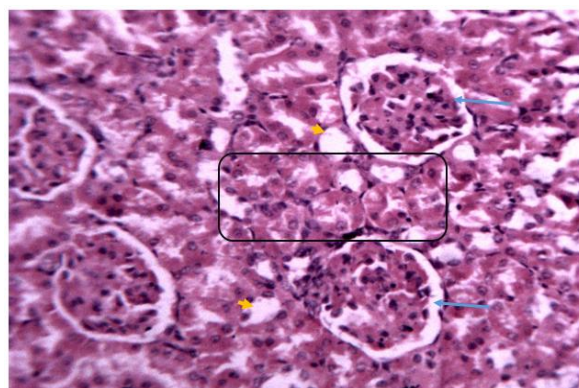


PLATE IV: Photomicrograph of the kidney of a Wistar rat with a kidney stone (Grp 4: *Cynodon dactylon* root (CDR)). Note near-normal glomeruli (arrows) except for a few slightly expanded glomerular spaces, more fairly visible tubules (rectangle) and vacuolations (arrowheads). H & E Stain. x250.

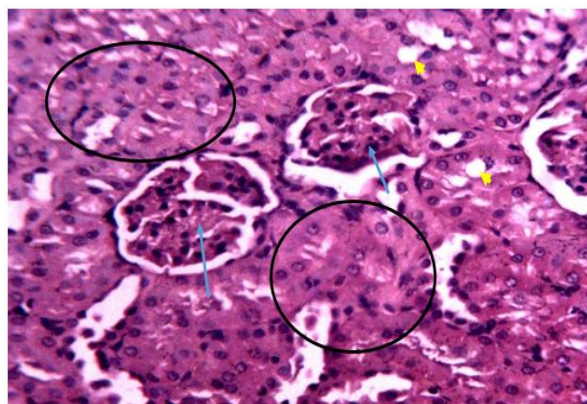


PLATE V: Photomicrograph of the kidney of a Wistar rat with a kidney stone (Grp 5: *Laportea* leaves (LL)). Note the mildly shrunken glomeruli (arrows), scanty vacuolations (arrow heads) and areas of necrosis (circled areas). H & E Stain. x250.

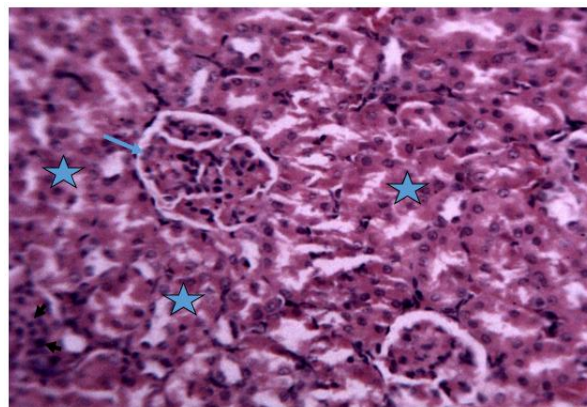


PLATE VI: Photomicrograph of the kidney of a Wistar rat with a kidney stone (Grp 6: MIL + CDR + LL). Note the glomeruli with almost normal outlines (arrows), regular tubular outlines with only a little hypercellularity (star/asterisk) and mild mononuclear cellular infiltration (arrowheads). H & E Stain. x250

GC- MS Result for Plant Sample Analysis: *Mangifera indica* leaves, *Cynodon dactylon* roots and *Fleurya aestuan*

Plants Sample	PK	RT	AREA	LIBRARY ID	QAUL
<i>Mangifera indica</i> leaves	1	7.708	57.48	(-)-Carvone	97
	2	11.688	0.89	Caryophyllene	99
	3	13.232	0.66	Isopropyl	97
<i>Cynodon dactylon</i> roots	1	7.625	57.35	Carvone	97
	2	16.159	10.52	Carotol	91
	3	16.841	8.25	Apiol	89
<i>Fleurya aestuan</i>	1	7.555	9.10	Carvone	93
	2	14.327	9.24	Dodecanoic acid	98
	3	26.689	7.75	9-Octadecenoic acid (Z)-	99

In the GC-MS result of *Mangifera indica* leaves, the following compounds were identified: carvone with pk area of 57.48 and retention time of 7.708, followed by Caryophyllene with pk area of 0.89 and retention time of 11.688 and Isopropyl with peak area of 0.66 and retention time of 13.232.

On the other hand, *Cynodon dactylon* roots GC-MS results revealed carvone with a peak area of 57.35 and retention time of 7.625, followed by carotol with a pk area of 10.52 and retention time of 16.159, and Apiol with a peak area of 8.25 and retention time of 16.841.

Fleurya aestuan leaves showed Carvone with pKa of 9.10 and retention time of 7.555, then Dodecanoic acid with pKa of 9.24 and retention time of 14.327, and 9-Octadecenoic acid (Z)- with pk area of 7.75 and retention time of 26.689

DISCUSSION

The present study demonstrates that ethanolic extracts of *Mangifera indica* leaves, *Cynodon dactylon* roots, and *Fleurya aestuan* leaves significantly reduced ethylene glycol-induced kidney stones in Wistar rats.

The result of qualitative phytochemical screening of *Mangifera indica* leaves in Table 1 is similar to the findings of (Charles et al., 2021), where flavonoids, alkaloids and phenols were present but saponins, glycosides and terpenoids were absent. The qualitative phytochemical findings of *Cynodon dactylon* roots are similar to those of (Madhan et al., 2018), which revealed the presence of alkaloids, tannins, glycosides and phenols but the absence of saponins. On the other hand, *Fleurya aestuan* leaves qualitative phytochemical screening correlated with that of (Nashir et al., 2016), where alkaloids, tannins, flavonoids, glycosides and terpenoids were present, but saponin was absent.

From the result of phytochemical screening obtained in Table 2, the highest concentration seen in flavonoids and phenols in *Mangifera indica* leaves and *Cynodon dactylon* roots is particularly notable. Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants, which have been shown to exert potent antioxidant activity against the superoxide radical (Temitope et al., 2012). Flavonoids are well known for their litholytic properties, i.e., their ability to disintegrate calcium oxalate stones, and their antioxidant ability, which protects renal cells from oxidative damage. The highest concentration of flavonoids in

Mangifera indica seen in this research work is similar to the finding of (Atara *et al.*, 2023). The result of this study also correlates with previous studies by (Asare *et al.*, 2021), who had earlier reported the highest concentration of flavonoids and phenols in *Mangifera indica* in their findings. The significantly higher level of phenol in *Cynodon dactylon* is similar to the findings of (Samira *et al.*, 2020), where phenol was identified in a higher quantity, suggesting strong antioxidant and anti-inflammatory effects, which are essential in managing kidney stones in the urinary tract (Samira *et al.*, 2020). In this study, in Table 3, the ethylene glycol-induced group exhibited a decline in body weight during the final week, from day 21 to day 28. The weight loss in this group suggests a sign of renal impairment and metabolic distress caused by the accumulation of calcium oxalate induced by Ethylene glycol. This finding is similar to that of (Olufunsho *et al.*, 2015) and that of (Venkatesan *et al.*, 2023). The significant improvement in body weight observed in group 3 (treated with *Mangifera indica* leaf extracts) and group 4 (treated with *Cynodon dactylon* root extract) suggested that this therapeutic effect is likely due to the high flavonoid and phenol content observed in Table 2. Group 6 (treated with all three plant extracts, i.e., *Mangifera indica*, *Cynodon dactylon* and *Fleurya aestuan*) showed steady and healthy weight gain; this suggests a synergistic effect, where the phytochemicals from all the plants work together to reduce the toxic effects caused by ethylene glycol more effectively than the individual extracts.

In Table 4, the highest serum levels of urea and creatinine observed in the ethylene glycol-treated group are similar to the findings of (Venkatesan *et al.* 2023), suggesting significant renal damage; this is because serum urea and creatinine are waste products normally filtered by the kidneys. Serum urea and creatinine are strong Biochemical markers for kidney health; ethylene induces stones and causes tubular obstruction, which prevents the kidneys from filtering out waste products, leading to the high levels observed in Table 3 in the ethylene-induced group (negative group). The decrease in the levels of these biochemical markers (urea, serum urea and calcium) observed in the groups treated with *Mangifera indica* leaves, *Cynodon dactylon* and *Fleurya aestuan* compared to the negative control group confirms the anti-urolithiatic potential of these plant extracts. The combination of the three plant extracts in group 6, which almost restored serum urea and creatinine to normal levels, suggests a strong synergistic effect of the formulations compared to the individual plant extracts in group 3. Group 4 was treated with only *Mangifera indica* leaf extracts, group 4. treated with only *Cynodon dactylon* root extracts, and group 5. treated with only *Cynodon dactylon* leaf extracts. The synergistic effects observed in group 6 suggest that the various phytochemicals, such as flavonoids from *Mangifera indica* leaves and phenols from *Cynodon dactylon* roots, identified in Table 1, work through multiple mechanisms to dissolve stones or protect against renal damage.

From the result of the histopathological examination, which offers direct morphological evidence of the therapeutic effects of the plant extracts against ethylene glycol-induced kidney stones in Wistar rats, Plate I (histopathology of kidney from positive control group) showed regular renal architecture with intact glomeruli and renal tubules. This confirms the healthy physiological stage of the animals, which perfectly aligns with the body weight gain observed in Table 2 and the baseline serum biochemical parameters seen in Table 3, indicating optimal kidney function.

Plate II. (histopathology of kidney from negative control group) Correlates with previous studies (Venkatesan *et al.*, 2023), which showed generalised distortion of renal parenchyma, shrinkage/compression of glomeruli, dilated renal tubules, intratubular crystal/deposit, and focal areas of necrosis as characteristics and indicators of calcium oxalate kidney stones. This severe structural renal damage directly explains the significantly elevated serum urea, uric acid and creatinine levels observed in Table 3. Additionally, the extensive tissue damage and metabolic dysfunction are consistent with the body weight decline observed in this group during the last stages of the study in Table 2, indicating toxicity and sickness of the animals.

Plate III. (histopathology of kidney from rats treated with only *Mangifera indica* leaf extracts) Resulted in noticeable improvement in the renal architecture: normal glomeruli, moderately dilated renal tubules with vacuolations, but some crystals/deposits were still present. This correlates with the significant reduction in serum urea, uric acid, and creatinine observed in Table 3, as well as improved body weight in Table 2 of this group. The high flavonoid content of *Mangifera indica* seen in Table 1 likely contributed to this improvement through its antioxidant and anti-inflammatory properties.

Plate IV. (histopathology of kidney from rats treated with only *Cynodon dactylon* root extracts) showed normal glomeruli except for slightly expanded glomerular spaces and more clearly visible tubules. This indicates high protection of key functional units of the kidney, particularly the absence of widespread necrosis and also the absence of intratubular crystal/deposit. These improvements are in line with the significant reduction of serum urea, uric acid, and creatinine seen in Table 3 and also the maintenance of healthier body weight observed in Table 2. These achievements may be as a result of the rich phenolic content obtained in Table 1, due to its strong anti-inflammatory properties.

Plate V. (histopathology of kidney from rats treated with only *Fleurya aestuan* leaves extract) resulted in mild shrunken glomeruli, scanty vacuolations, and areas of necrosis. However, it is still better than the negative control group treated with only ethylene glycol. The mild shrinkage and necrosis observed in this group suggest a less protective effect of *Fleurya aestuan* leaves extract compared to *Mangifera indica* leaves and *Cynodon dactylon* roots extracts. This aligns with the lack of a significant impact of *Fleurya aestuan* on body weight in Table 2 and the relatively less reduction in some biochemical markers in Table 3, particularly for serum calcium.

Plate VI. (Histopathology of kidneys from rats treated with both *Mangifera indica*, *Cynodon dactylon*, and *Fleurya aestuan* leaf extracts). The kidney section of this group displayed glomeruli with almost normal outlines, regular tubular outlines with little hypercellularity and mild mononuclear infiltrations. This indicates an almost complete restoration of renal architecture with minimal signs of damage or inflammation. This provides visual evidence for synergistic effects when the three plant extracts are combined. The histological findings in this group perfectly support the optimal biochemical parameters, particularly urea and creatinine levels, which were restored to almost normal in Table 3, and healthy body weight gain in Table 2.

Mangifera indica leaf extract was subjected to Gas Chromatography - Mass Spectrometry.

(GC-MS) analysis, and 35 compounds were identified. From the compounds identified, the following with high peak area were selected for detailed interpretation. Carvone (57.48%) was similar to the findings of Shrama et al. (2017), where carvone was identified and recognized for its antioxidant, anti-inflammatory, and antimicrobial properties. Caryophyllene (0.89%) was also among the compounds identified, and this correlates with the findings of Adeyinka et al. (2005), where caryophyllene was identified at 7.49%. Caryophyllene is well known for its antioxidant activities (Adeyinka et al., 2005). Additionally, Isopropyl (0.66%) was also identified, which correlates with the findings of Adeyinka et al. (2005), where isopropyl was identified at (3.20), and it is well known for its antioxidant properties (Adeyinka et al., 2005).

From the above findings, the high concentration of carvone directly accounts for a significant portion of the high flavonoid and phenolic contents reported in Table 1, as the components exhibit antioxidant activities.

GC-MS studies of *Cynodon Dactylon* Root extracts showed that 21 compounds were identified, and the following compounds with high peak area were selected for interpretation. Carvone (57.37%), Carotol (10.52%), Apiol (8.25%), Hexadecanoic acid methyl ester (3.72%), 9-octadecenoic acid (2.99%). The identification of carvone in this study is similar to the findings of Lee et al. (2019); carvone is known for its antioxidant and anti-inflammatory potential (Lee et al., 2019). Hexadecanoic acid (3.72%) correlates with the findings of (Rawal & Sonawani 2017), where hexadecanoic acid was identified at 14.90%. Hexadecanoic acid is well known for its antioxidant and antimicrobial activities (Dikshita et al., 2021). Octadecenoic acid (2.99%) is similar to findings (Dikshita et al., 2021), where Octadecenoic acid was found at 18.51% and has potential anti-inflammatory activities (Yadav et al., 2017).

The GC-MS of *Cynodon dactylon* provides critical molecular insights. For instance, the high abundance of carvone correlates with high phenolic content (7.36% in Table 1), suggesting a significant reduction in elevated urea and creatinine levels in Table 3.

GC-MS analysis of ethanolic extracts of *Laportea* leaves successfully identified 27 compounds, with the most prominent, based on peak area, being Dodecanoic acid (9.24%), hexadecanoic acid methyl ester (8.97%), and Octadecenoic acid methyl ester (4.54%). Dodecanoic acid is similar to the findings of Kolade et al. (2024), where Octadecenoic acid methyl ester was identified at 1.30%, commonly known as lauric acid; this is a saturated fatty acid well known for its antimicrobial properties (Papageorgiou et al., 2018). Hexadecanoic acid methyl ester in this study correlates with the findings of Oguntimehin et al. (2017), where this compound was identified with a peak area of 4.55. Hexadecanoic acid methyl ester is well known for its Anti-inflammatory and antioxidant effects (Odo et al., 2022). Octadecenoic acid, with a peak area of 4.54%, is similar to the findings of Odo et al. (2022), where this compound is identified with a peak area of 1.30. This compound is well known for its Antioxidant and antimicrobial properties (Odo et al., 2022).

GC-MS of *Laportea* leaves extracts shows a significant reduction in serum urea and creatinine (Table 3), and improved renal histology with mildly shrunken glomeruli; its effects on body weight (Table 2) were not statistically significant.

Conclusion

This research successfully investigated the Effects of *Mangifera indica* (Mango) Leaves, *Cynodon dactylon* (Bermuda Grass) Roots and *Fleurya aestuans* (Laportea) Leaves Ethanolic Extracts on Ethylene Glycol-Induced Kidney Stones in Wistar Rats.

The quantitative phytochemical screening analysis in Table 1 established that these plants are rich in bioactive components (high flavonoid content in *Mangifera indica* and phenolic content in *Cynodon dactylon*), which are known for antioxidant, antimicrobial and anti-inflammatory properties. The GC-MS analysis further identified carvone as a dominant bioactive component in *Mangifera indica* and *Cynodon dactylon* leaf extracts. At the same time, fatty acid methyl esters were dominant in *Laportea* leaf extracts.

The massive elevation of biomarkers (serum urea, uric acid and creatinine) in the negative control group was significantly reversed by the plant extracts. Notably, these biomarkers were restored to almost normal levels in the combined extract (group 6), suggesting a powerful synergistic effect of *Mangifera indica* leaves, *Cynodon dactylon* roots and *Fleurya aestuans* in kidney stone dissolution.

The histopathology interpretations confirmed damage to renal parenchyma, necrosis and crystal deposits in the negative control group; the plant formulations, particularly the combination of the three plant extracts, successfully restored parenchyma and glomerular architecture.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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