

PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF ETHANOLIC EXTRACTS OF THE LEAVES OF *MANGIFERA INDICA* AND *FLEURYA AESTUANS* AND ROOTS OF *CYNODON DACTYLON*

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ABSTRACT

Traditional use of *Mangifera indica*, *Cynodon dactylon* and *Fleurya aestuans* for urinary ailments suggests bioactive phytochemicals that may underlie anti-urolithic effects. The objective of this research is to perform qualitative and quantitative phytochemical screening and GC-MS profiling of ethanolic extracts of the leaves of *Mangifera indica* (MIL) and *Fleurya aestuans* (FAL) as well as roots of *Cynodon dactylon* (CDR). Dried powders of each plant sample (500 g each) were extracted in 95% ethanol by maceration, concentrated and stored. Standard qualitative tests and quantitative colorimetric assays (alkaloids, flavonoids, tannins, glycosides, saponins, phenols, steroids, terpenoids) were performed in triplicate. GC-MS analysis was used to identify major volatile/semi-volatile constituents. Qualitative screening showed the presence of flavonoids, tannins, phenols, terpenoids and glycosides in all three extracts; alkaloids were present only in *Mangifera indica* leaves. Quantitative results (mean $\pm$ SEM) showed the highest flavonoid content in *Mangifera indica* leaves (9.33 ± 0.42) and the highest phenolic content in *Cynodon dactylon* roots (7.36 ± 0.35). GC-MS of *Mangifera indica* leaves identified (-)-carvone as the dominant peak (57.48% area); *Cynodon dactylon* roots GC-MS also showed a dominant carvone peak (57.35% area). *Fleurya aestuans* leaves chromatograms were dominated by fatty acid methyl esters. Several terpenes and fatty acids were detected. The three plants present distinct phytochemical fingerprints; the abundance of carvone in *Mangifera indica* leaves, *Cynodon dactylon* roots, and *Fleurya aestuans* leaves provides a chemical rationale for further bioactivity testing and for the anti-urolithic activity observed in vivo.

Keywords: *Mangifera indica*, *Cynodon dactylon*, *Fleurya aestuans*, phytochemical screening, GC-MS, carvone, fatty acid methyl esters.

INTRODUCTION

Kidney stones are hard deposits of minerals and acid salts that stick together in concentrated urine. The most common crystalline minerals found in kidney stones are calcium oxalate, calcium phosphate, uric acid and struvite (Aishwarya *et al.*, 2021). 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 2-3 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 and Revikumar, 2011).

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 pharmacological limitations and side effects with long-term use and are not effective in completely removing the stones (Aishwarya *et al.*, 2021).

Studies have reported *Mangifera indica* fruit (mango) to possess anti-diabetic, anti-oxidant, anti-viral, cardiogenic, hypotensive, and anti-inflammatory properties (Barreto *et al.*, 2008). In African traditional medicine and in particular among Yoruba, Hausa and Igbo communities in Nigeria, various parts of *Mangifera indica* trees are used in the treatment of different human and veterinary diseases, including malaria (Ene *et al.*, 2010), dysentery, cough and typhoid fever infection (Alo *et al.*, 2012).

Cynodon dactylon, also called Bermuda grass, is claimed to have anti-diabetic, antimicrobial, hypolipidemic, anti-inflammatory, and anti-emetic properties. The plant is diuretic and used as a remedy for urinary infections, kidney stones and congestion. The diuretic effect of *C. dactylon* was attributed to the presence of flavonoids (Abolfazl *et al.*, 2011).

Fleurya Aestuans (Laportea) is a tropical rainforest weed (Cheryl, 2007). Its phytochemical constituents include saponins, tannins, flavonoids, cardiac glycosides, alkaloids and phenolic compounds (Claudia *et al.*, 2006). Laportea is commonly used to treat laryngitis, arthritis, anemia, hay fever, kidney diseases and urinary problems, diabetes, bronchitis and filariasis (Focho *et al.*, 2009). Medicinal plants are widely used in traditional systems to prevent or treat stones, but scientific chemical profiling is often lacking. Chemical characterization (phytochemical assays and GC-MS) provides a foundation for pharmacological validation and identification of candidate bioactive compounds. This study sought to profile three plants commonly used in Nigerian traditional medicine for urinary disorders: *Mangifera indica* (leaves), *Cynodon dactylon* (roots) and *Fleurya aestuans* (leaves) to identify compounds that might mediate anti-urolithic or nephroprotective effects.

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: MIL — NDA/BIOH202509; CDR — NDA/BIOH202508; FAL — NDA/BIOH202510).

Extraction

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

Qualitative Phytochemical Screening

Alkaloids

Dragendorff's reagent was used, and the method described by Harborne was adopted. Grinded and powdered sample (0.2g) was extracted with 95% ethanol in a Soxhlet extractor for six hours and the ethanolic extract evaporated to dryness using a vacuum evaporator at 45°C. The residue was redissolved in 5 ml 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 1 g 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 and Evans (2009) was adopted. 0.5 g of each plant sample was dissolved in 5ml 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 (H₂SO₄) 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

1g of each plant sample was macerated with 20 ml of ethanol and 20% sulfuric acid (H₂SO₄) (1:1 v/v). 1 ml of the filtrate was added to 5 ml of 60% H₂SO₄. After 5 min, 5 ml of 0.5% formaldehyde in

60% H₂SO₄ 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

1g 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, along with 2.5 ml of concentrated H₂SO₄, 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 - 25µg 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 Polyphenols

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 filtered solution was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl₃ 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 H₂O and 8 ml of 12.5% lead acetate were added, mixed and filtered. 50ml of the filtrate was transferred into another 100 ml flask, and 8ml of 47% Na₂HPO₄ was added to precipitate excess Pb²⁺ 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

10ml 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 495nm.

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 500g was macerated in 95% ethanol at room temperature for 48hours. The entire process was repeated twice for a period of 24hours 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.

DATA ANALYSIS

Data are mean ± SEM. Differences across samples were tested by one-way ANOVA with Dunnett's multiple comparison test; significance at p < 0.05.

RESULTS

Qualitative phytochemical screening

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	+	+	+

Presence = +, Absence = -

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

Quantitative Phytochemical Analysis

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.

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

GC- MS Analysis

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 pk area of 9.10 and retention time of 7.555, then Dodecanoic acid with pk area 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

This study provides comparative phytochemical and GC-MS profiling of ethanolic extracts from *Mangifera indica* leaves, *Cynodon dactylon* roots and *Fleurya aestuan* leaves, revealing distinct chemicals that underlie their traditional use in kidney stone treatment.

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 absent of saponins, glycosides and terpenoids. 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, qualitative phytochemical screening of *Fleurya aestuan* leaves correlates 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., 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 is essential in managing kidney stones in the urinary tract (Samira *et al.*, 2020). *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%), which is 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, where 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 the findings of 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).

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 the 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 Oguntimhin *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).

Conclusion

The quantitative phytochemical screening analysis 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 their 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 *Fleurya aestuan* leaf extracts. These profiles provide a chemical basis for the traditional use of these plants in urinary disorders.

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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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