



Article Info

Received: 23rd May 2025Revised: 27th June 2025Accepted: 3rd July 2025

¹Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti.

²Department of Medical Laboratory Science, Faculty of Health Sciences, Al-Hikmah University, Ilorin, Kwara State, Nigeria.

³Department of Immunology, Faculty of Nursing and Allied Health Sciences, University of Abuja.

*Corresponding author's email:

rtbaba@alhikmah.edu.ngomotayo.rasheedat@gmail.com Phone:[07065391534](tel:07065391534); [09026144501](tel:09026144501)

Cite this: CaJoST, 2025, 2, 282-290

Investigation of the Phytochemical Constituents of *Euphorbia hirta* and *Senna alata* using Qualitative, Quantitative and Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

Rasheedat T. Baba^{1,2}, Bernard O. Oluboyo¹, Adebimpe H. Egbebi¹, Mariam Aladodo³, Zainab A. Dangana², Zainab K. Muhammad², Hawau B. Sowole², Husseina U. Sani², Firdaus Yakubu²

Medicinal plants have been central to traditional medicine due to the abundance of bioactive compounds they contain. *Euphorbia hirta* and *Senna alata* are notable examples of medicinal plants widely used in ethnomedicine and recognized for their proven pharmacological properties. This study aimed at investigating the phytochemical constituents of *Euphorbia hirta* and *Senna alata* using qualitative screening, quantitative analysis, and Gas Chromatography–Mass Spectrometry (GC-MS). Methanolic and aqueous extracts of the plants were prepared using soxhlet extraction and maceration techniques respectively and the qualitative and quantitative analysis were carried out using standard techniques and GC-MS. Qualitative analysis revealed the presence of key bioactive compounds such as alkaloids, flavonoids, saponins, and tannins in both plants. Quantitative analysis confirmed alkaloids as the most abundant phytochemical, particularly in *Senna alata*, followed by saponins and flavonoids. GC-MS profiling identified a wide range of pharmacologically active compounds, including γ-Tocopherol, oleic acid, and n-Hexadecanoic acid in *S. alata*, and quercetin, 9-Octadecenal, and eicosatrienoic acid methyl ester in *E. hirta*. The findings demonstrate that methanol is a more effective solvent for extracting a broader spectrum of phytochemicals, and that *Senna alata* exhibits a richer and more diverse chemical profile than *Euphorbia hirta*. These results support the potential of both plants, particularly *S. alata*, as sources of natural compounds for therapeutic and pharmaceutical applications.

Keywords: Phytochemical constituents, *Euphorbia hirta*, *Senna alata*, Methanolic and Aqueous.

1. Introduction

Medicinal plants have long served as the cornerstone of traditional medicine across various cultures, owing to their rich repertoire of bioactive compounds (Halder & Jha, 2023). Among the vast array of phytotherapeutic agents, *Euphorbia hirta* and *Senna alata* stand out due to their widespread ethnomedicinal usage and well-documented pharmacological activities (Anigboro *et al.*, 2022). *Euphorbia hirta* (family: Euphorbiaceae), commonly known as asthma weed, is a pantropical herb often used in traditional medicine for the treatment of asthma, bronchitis, diarrhea, and skin ailments (Kumar *et al.*, 2024). Similarly, *Senna alata* (family: Fabaceae), also known as the candle bush, is widely used in the treatment of fungal infections, constipation, and skin diseases (Oladeji *et al.*, 2020). Phytochemical screening provides a

qualitative assessment of plant secondary metabolites, which are crucial for understanding the therapeutic potential of medicinal plants. These secondary metabolites often work synergistically to produce the desired biological effects (Dubale *et al.*, 2023). Gas Chromatography–Mass Spectrometry (GC-MS) is a powerful analytical tool employed to elucidate the volatile and semi-volatile organic compounds within plant extracts. Through GC-MS, numerous bioactive constituents can be identified in *E. hirta* and *S. alata*. Understanding the phytochemical constituents and chemical composition of these plants through GC-MS analysis not only validates their traditional uses but also paves the way for novel drug discovery and development (Ibrahim *et al.*, 2021). Additionally, such comparative studies enhance the

pharmacognostic profiling of medicinal plants, fostering their integration into evidence-based complementary and alternative medicine (Ibrahim et al., 2021). Therefore, the current study aimed at conducting a comprehensive phytochemical screening and GC-MS analysis of methanol and aqueous extracts of *Euphorbia hirta* and *Senna alata*. This approach will provide scientific validation of their ethnomedicinal applications and contribute to the broader understanding of their therapeutic potential (Thamer & Thamer, 2023).

2. Materials and Methods

2.1 Plant Collection, Authentication and Pulverization of the Plant Leaves

Fresh leaves of *Euphorbia hirta* and *Senna alata* were collected from Kwara State stadium Ilorin, Nigeria. The plants were identified and authenticated at the herbarium unit of the Department of plant biology, Faculty of life sciences, University of Ilorin, Kwara State, where they were given the herbarium voucher number **UILH/001/1511/2024** and **UILH/001/1059/2024** respectively. Leaves of *Euphorbia hirta* and *Senna alata* were washed thoroughly separately and air dried in shade for two weeks. Dried leaves were ground into a coarse powder using an industrial blender.

2.2 Plant Extraction

2.2.1 Preparation of the plant extract

Methanol and aqueous extracts of *Euphorbia hirta* and *Senna alata* leaves were prepared using Soxhlet extraction and maceration methods, respectively (Abubakar & Haque, 2020). For the methanol extracts, 100 g of powdered leaves were placed in Soxhlet apparatus thimbles and extracted with 500 ml of methanol through continuous solvent reflux until exhaustion. The resulting extracts were concentrated using a rotary evaporator at 50 °C and stored at 4 °C. For the aqueous extracts, 100 g of ground leaves were soaked in 200 ml of distilled water for 72 hours at room temperature with occasional stirring. After filtration, the extracts were frozen at -20 °C and lyophilized at -50 °C under vacuum to obtain dry powders. The percentage yields of the extracts were calculated and the extracts were stored at 4 °C for further microbiological and phytochemical analyses.

2.3 Phytochemicals of Plant Extracts

2.3.1 Qualitative Analysis

Qualitative analysis was performed to identify the presence of secondary metabolites in both aqueous and methanolic extracts of *Euphorbia hirta* and *Senna alata*. The phytochemical analysis was performed using standard laboratory techniques for qualitative determination.

2.3.1.1 Test for phenol: This was carried out by the method of Dubale et al., (2023): Exactly 5 ml of each extracts was dissolved in 5 ml of distilled water and few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

2.3.1.2 Test for Alkaloids: This was carried out by the method of Misiurek et al., (2023): Exactly 2 ml of each extract was collected using syringe and dispensed into different labelled test tubes. The test tubes were heated for 2 minutes and 5 ml of hydrochloric acid (HCl) was added into each tube, mixed and heated again and allowed to cool. 2 drops of Meyer's reagent was added to each of the test tubes and white precipitate was observed which showed the presence of alkaloids.

2.3.1.3 Test for Cardiac glycosides: This was carried out by the method of Joubert (2023): The extract (2 ml) of each extract was collected into test tubes and 5 ml of glacial acetic acid was added, followed by 2 ml of FeCl₃ and 2 ml of concentrated ferric acid. A brown ring formation at interphase of the mixture indicated the presence of deoxy sugar characteristics of cardiac glycosides.

2.3.1.4 Test for Saponins: Foam test was performed to test the presence of saponins. To the 2ml of each of the extract, added 6ml of water in a test tube and was shaken vigorously, then observed for the formation of persistent foam that confirms the presence of saponins (Bakir et al., 2022).

2.3.1.5 Test for flavonoids: Exactly 5 ml of each of the extracts was collected into labeled test tube. 10 ml of distilled water, 5 ml of dilute ammonium hydroxide (NH₄OH) and few drops of tetraoxosulphate (IV) acid (H₂SO₄) were added in the test tube. A yellow colouration was observed which showed the presence of flavonoids (Ayele et al., 2022)

2.3.1.6 Test for Tannins: This was carried out by the method of Zhang et al., (2023). The extract (1 ml) was collected into labeled test tubes. Then, 1 ml of (10 %) ferric chloride (FeCl₃) was added to the test tubes. Dirty green precipitate was observed which showed the presence of tannins.

2.3.1.7 Test for Terpenoid: Terpenoid presence was determined through Salkowski test. 2 ml of chloroform was mixed thoroughly with each of the extract filtrate. 3 ml of concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids (Godlewska et al., 2022).

2.3.2 Quantitative Analysis

2.3.2.1 Determination of Total Phenols

This was determined using the Folin-Ciocalteu method (Lawag et al., 2023).

With a little modification, the Folin–Ciocalteu technique was used to measure the content of phenolic compounds in the extracts. In a volumetric

flask (10 mL), 50 μ L of the sample extracts were combined with 0.5 mL of (1 N) Folin–Ciocalteu reagent. 2.5 mL of 5% sodium carbonate was added after 5 min, and distilled water was used to get it up to the volume of 4 mL. The tubes were incubated for 40 min at room temperature in a dark area with a series of gallic acid standard solutions (2.5, 5, 7.5, 10, and 12.5 mg/mL) made in the same way. The absorbance was measured at 760 nm. The total phenol content was expressed as milligrams of gallic acid equivalent per gram of dried extract. The same method was used to make a blank reagent that was devoid of extracts.

2.3.2.2 Determination of Tannins

The total tannin content was assessed by the standard protocol of Logeswaran & Vadivel (2024) with little modifications as follows: 0.5 mL of the sample extract was diluted with 80% ethanol. From the diluted sample, 0.1 mL was added to 2 mL of FolinCiocalteu reagent. After 8 mins, 7.5 mL of 7% sodium carbonate solution was added and incubated for 2 hours. The absorbance was measured at 760 nm and the tannin content was estimated using tannic acid curve as the standard

2.3.2.3 Determination of Flavonoid

Approximately 0.25 g of the sample was dissolved in 1 ml distilled water; 0.15ml of 5% NaNO₂ solution, 0.15ml of freshly prepared aluminum chloride (AlCl₃) and 2.0ml of 1M NaOH solutions were added. The mixture was allowed to stand for 5 min and absorbance measured at 510 nm on a SpectrumLab70 spectrophotometer. The result was expressed as quercetin equivalents (QE) (Ayele *et al.*, 2022).

2.3.2.4 Determination of Saponin

The saponin content was determined using spectrophotometric method described by (Sodimu *et al.*, 2024). About 2 g of the sample was weighed into a beaker and 150ml of isobutyl alcohol (but-2-ol) was added. The mixture was filtered through No 1 Whatman filter paper into a beaker containing 20ml of 40% magnesium carbonate (MgCO₃) solution. Approximately 1 ml of the solution was transferred into volumetric flask; 2 ml iron (III) chloride (FeCl₃) solution was added and made up to mark with distilled water. This was allowed to stand for 30 min for the colour development and absorbance was read at 380 nm on a SpectrumLab70 spectrophotometer.

2.3.2.5 Determination of Alkaloid

5 g of the sample was weighed into a beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 min. This was filtered and the extract was concentrated on a waterbath to one quarter of the original volume. Ammonium hydroxide solution was added drop wise to the extract until the

precipitation was completed. The precipitate was collected, washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed (Obasi and Obasi, 2021).

2.4 Phytochemical analysis using Gas chromatography-mass spectrometry (GC-MS)

This analysis was carried using a Varian 3800/4000 gas chromatograph mass spectrometer equipped with an Agilent capillary column employing the following conditions: fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m \times 250 μ m, film thickness 0.25 μ m), interfaced with 5675C Inert MSD with Triple-Axis detector. Nitrogen gas was used as carrier gas and was adjusted to column velocity flow of 1.0 ml/min. Other GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 16.2 psi; out time, 1.8 mm; and 1 μ L injector in split mode with split ratio 1:50 with injection temperature of 300 °C. The column temperature started at 36 °C for 5 min and changed to 150 V at the rate of 4 °C/min. The temperature was raised to 250 °C at the rate of 20 °C/min and held for 5 min. The total elution was 43.50min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. ChemStation software provided by supplier was used to control the system and to acquire the data.

2.4.1 Identification of compounds

Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of Hibiscus asper fraction obtained were compared with the standard mass spectra of known components stored in NIST library (NIST08).

2.5 Statistical Analysis

All the data were statistically analyzed by determining the p value through the t-test and the significant level was considered as <0.05. All the data were analyzed using SPSS, version 25.

3. Results and Discussion

3.1 Results

3.1.1 Extraction

The percentage of the extracts are given in Table 1

3.1.2 Phytochemical Analyses

The phytochemical analysis revealed the presence of flavonoids, Alkaloid, Tannin, saponin, cardiac glucoside, phenol, Quinone and Terpenoids... (Table 2). The quantitative phytochemical analysis are presented in Table 3 and 4.

Table 1: Percentage yield of *Euphorbia hirta* and *Senna alata* extracts

Plants	Solvents	Weight of plants/g	Weight of extracts/g	Percentage yield %
<i>Euphorbia hirta</i>	Aqueous	100	5.2	5.2
	methanol	100	6.4	6.4
<i>Senna alata</i>	Aqueous	100	6.0	6.0
	methanol	100	7.9	7.9

Table 2: Qualitative phytochemical analysis of Aqueous and Methanolic extracts of *Euphorbia hirta* and *Senna alata* plants

	<i>Senna alata</i>		<i>Euphorbia hirta</i>	
	Aqueous	Methanol	Aqueous	Methanol
Flavonoids	++	++	++	++
Tannins	++	++	++	+++
Cardiac glycosides	+++	+++	-	-
Alkaloids	++	++	++	+++
Terpenoids	+	+	-	-
Phenols	-	+	-	-
Quinone	+	+	-	-
Saponin	++	++	+++	++

Keys: + = positive = Negative

Table 3: Quantitative phytochemical analysis of aqueous and methanol extracts of *Euphorbia hirta*

Extract	Composition		t-value	p-value
Phytochemicals	Aqueous (Mean \pm SD)	Methanol (Mean \pm SD)		
Total Saponin Content (μ g/g)	43.8 \pm 0.021	30.4 \pm 0.035	420.572	0.000**
Total Tannin Content (μ g AAE/g)	12.8 \pm 0.015	13.2 \pm 0.025	-17.754	0.003**
Total Flavonoid Content (μ g RE/g)	37.5 \pm 0.026	11.7 \pm 0.017	2236.944	0.000**
Total Alkaloids Content (μ g/g)	46.4 \pm 0.020	42.0 \pm 0.030	173.646	0.000**

Key: % = Percentage ** mean significance at p-value < 0.05

Table 4: Quantitative Phytochemical Composition of the *Senna alata* extracts

SA Extract	Composition		t-value	p-value
Phytochemicals	Aqueous (Mean \pm SD)	Methanol (Mean \pm SD)		
Total Phenol Content (mg GAE/g)	-	8.3 \pm 0.567	4.307	0.050
Total Saponin Content (μ g/g)	32.8 \pm 0.036	40.0 \pm 0.000	-344.916	0.000**
Total Tannin Content (μ g AAE/g)	43.0 \pm 0.012	52.5 \pm 0.010	-4345.000	0.000**
Total Flavonoid Content (μ g RE/g)	38.0 \pm 0.040	40.3 \pm 0.023	-85.625	0.000**
Total Alkaloids Content (μ g/g)	56.3 \pm 0.015	44.9 \pm 0.030	-680.600	0.000**

Key: % = Percentage ** mean significance at p-value < 0.05

3.1.3 GC-MS Analysis

The chromatograms from the GC-MS analyses are shown in Figures 1 and 2 while important parameters are presented in Tables 5 and 6.

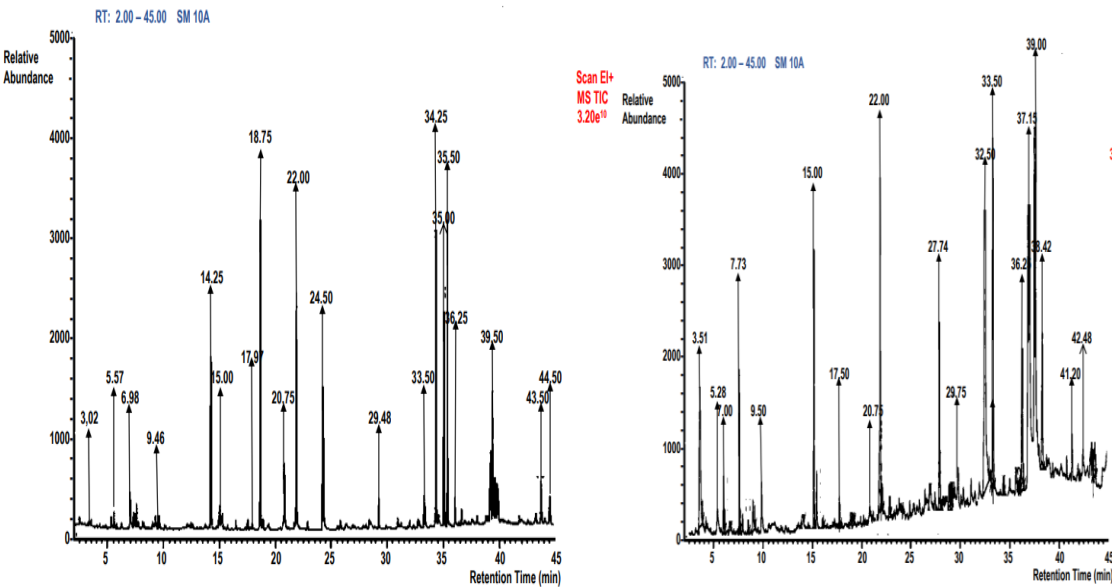


Figure 1: GCMS chromatogram of methanol and aqueous extract of *Euphorbia hirta*

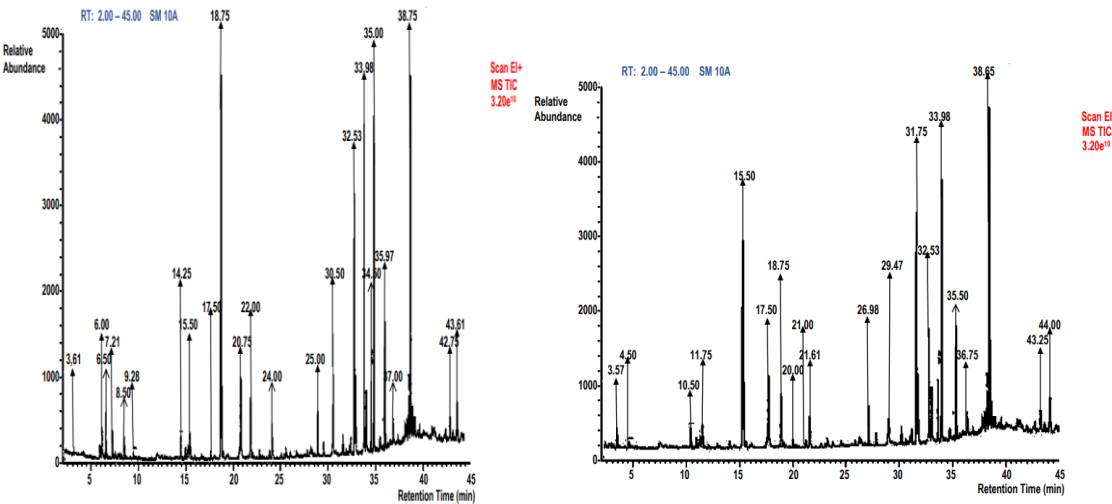


Figure 2: GCMS chromatogram of methanol and aqueous extract of *Senna alata*

Table 5: Gas chromatography-mass spectrometry of *Euphorbia hirta*

Peak #	RT	Compound Detected	Mol. Formular	MW	Peak Area %	Comp %wt	m/z
1	5.57	1,2,3-Benzenetriol	C ₆ H ₆ O ₃	126	1.03	1.93	52, 80, 126
2	6.98	1-(3-Dimethylaminophenyl)ethanol	C ₁₀ H ₁₅ NO	165	1.71	2.33	122, 164, 165
3	14.25	Glycol aldehyde dimer	C ₆ H ₈ O ₄	120	7.69	15.73	44, 93, 120

4	15.00	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	8.67	12.91	43, 73, 256
5	17.50	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	1.02	4.75	42, 73, 146
6	20.75	Phytol	C ₂₀ H ₄₀ O	296	0.64	1.12	43, 71, 296
7	22.00	1,2-Cyclohexanedicarboxylic acid, diethyl ester	C ₁₂ H ₂₀ O ₄	228	10.20	6.97	41, 81, 228
8	27.74	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	6.12	11.73	41, 67, 280
9	29.25	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	2.04	9.24	43, 73, 284
10	32.50	Quercetin	C ₁₅ H ₁₀ O ₇	302	9.18	5.82	43, 74, 302

Table 6: Gas chromatography-mass spectrometry of *Senna alata*

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp %wt	m/z
1	3.21	1-Butanol, 3-methyl-	C ₅ H ₁₂ O	88	0.32	1.02	42, 55, 88
2	6.00	1-Hexanol, 2-ethyl-	C ₈ H ₁₈ O	130	1.27	1.73	41, 57, 130
3	6.50	Carbamic acid, phenyl ester	C ₇ H ₇ NO ₂	137	1.91	2.52	43, 94, 137
4	7.21	Xylitol	C ₅ H ₁₂ O ₅	152	0.96	1.21	43, 61, 152
5	8.50	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	0.80	1.04	77, 135, 150
6	3.57	1-Butanol, 3-methyl-	C ₅ H ₁₂ O	88	1.57	2.64	42, 55, 88
7	4.50	β-D-Glucopyranose, 1,6-anhydro-	C ₆ H ₁₀ O ₅	162	0.79	1.98	43, 60, 162
8	10.50	α-Methyl-D-mannopyranoside	C ₇ H ₁₄ O ₆	194	1.97	2.88	77, 131, 194
9	11.75	1,4-Bis(trimethylsilyl)benzene	C ₁₂ H ₂₂ Si ₂	222	2.05	4.54	73, 207, 222
10	16.50	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	11.42	13.12	43, 73, 256

3.2 Discussion

The extraction yields of the medicinal plants investigated in this study demonstrated notable variability, with the highest yield obtained from the

methanol extract of *Senna alata* (7.9%), while the aqueous extract of *Euphorbia hirta* yielded the least. This variation is primarily attributable to the nature of the solvent used, which significantly affects the solubility and extractability of phytochemicals. Polar

solvents such as water are more selective for hydrophilic compounds, whereas solvents like methanol, with intermediate polarity, can extract a broader range of both polar and non-polar constituents, resulting in higher yields. These findings align with previous studies by Ghaffar & Perveen, (2024), who reported similar solvent-dependent variations in extractive values of medicinal plants. Qualitative and quantitative phytochemical analyses revealed the presence of key secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids in varying concentrations. Alkaloids were notably abundant in both plants, particularly in *Senna alata*, supporting earlier observations that these compounds are central to the plant's ethnopharmacological uses (Osunga et al., 2024). Flavonoids and tannins, known for their diverse pharmacological properties including antioxidant, anti-inflammatory, and cytoprotective activities were more prominent in methanol extracts. This supports earlier assertions by Caratti, (2023) and Rahim et al. (2023) that solvent type significantly impacts the recovery of bioactive polyphenols. GC-MS profiling of *Senna alata* methanol extract identified a wide array of compounds including saturated and unsaturated fatty acids, alcohols, esters, sterols, and tocopherols. Notably, n-Hexadecanoic acid (palmitic acid), 9,12-octadecadienoic acid (linoleic acid), oleic acid, and stearic acid were among the major constituents. These compounds are well-documented for their multiple pharmacological functions such as antioxidant, anti-inflammatory, cytotoxic, and membrane-stabilizing effects (Adegoke & Olakayode, 2024; Goh et al., 2024). Phytol, a diterpene alcohol found in moderate concentrations, has been associated with a broad spectrum of bioactivities including antimalarial, antioxidant, and cytostatic effects. The presence of phytol in *Senna alata* complements previous reports by Santhosh et al. (2022), who identified phytol as a key contributor to the therapeutic properties of several plant-based remedies. Additionally, vitamin E derivatives such as γ -tocopherol and α -tocopheryl acetate were detected in significant amounts. These lipophilic antioxidants are known not only for their free radical scavenging activity but also for their role in enhancing membrane stability and modulating inflammatory responses (Ma et al., 2023). Their abundance in *Senna alata* may support its use in managing oxidative stress-related conditions. Steroidal phytochemicals such as stigmasterol and campesterol were also identified. These plant sterols have been shown to possess cholesterol-lowering, anti-inflammatory, and immune-modulatory properties (Kato et al., 2023). Although their direct pharmacological actions may be moderate, they are often involved in synergistic interactions that enhance the overall efficacy of botanical formulations. Comparatively, *Euphorbia hirta* exhibited a different phytochemical profile, with a

stronger presence of polyphenolic compounds such as quercetin, pyrogallol, and catechins. These compounds are potent antioxidants and are often associated with protective effects against degenerative diseases, including cardiovascular disorders and certain types of cancer (Muscolo et al., 2024). The diversity in bioactive profiles between *Senna alata* and *Euphorbia hirta* suggests that their therapeutic applications may differ in target and potency. While *Senna alata* appears to possess a phytochemical composition skewed toward lipid-soluble compounds with potential for membrane-targeting and anti-inflammatory actions, *Euphorbia hirta* is richer in water-soluble phenolics, which may contribute more significantly to antioxidant and cytoprotective mechanisms. These findings underscore the importance of solvent selection in phytochemical research and highlight the potential of these medicinal plants as sources of pharmacologically relevant compounds. Moreover, the identification of multiple classes of bioactives supports their traditional use in ethnomedicine and suggests a multi-target approach in their therapeutic efficacy.

4. Conclusion

This study examined the extraction yields and phytochemical profiles of *Senna alata* and *Euphorbia hirta* using methanol and water as solvents. Methanol extracts produced higher yields and contained more diverse bioactive compounds including alkaloids, flavonoids, fatty acids, sterols, and tocopherols. GC-MS analysis revealed key compounds such as palmitic acid, linoleic acid, oleic acid, and phytol, which are known for their antioxidant, anti-inflammatory, and therapeutic properties. *Senna alata* was particularly rich in lipid-soluble compounds, while *Euphorbia hirta* had higher levels of polyphenolics. These differences suggest distinct therapeutic potentials: *Senna alata* may be more effective in treating inflammation and membrane-related conditions, while *Euphorbia hirta* may better address oxidative stress. Overall, the findings highlight the importance of solvent selection in phytochemical extraction and support the traditional medicinal use of both plants, providing a scientific basis for their further pharmacological development.

Authors' contributions

Baba Rasheedat carried out the research; Dangana Zainab Aminu, Muhammad Zainab Kende, Sowole Hawau Bolaji Sani Hussaina Usman and Yakubu Firdausi collected samples and prepared them for extraction; Oluboyo Bernard Oluwapelumi supervised the project; Egbebi, Adebimpe Helen co-supervised the project Aladodo Maryam and Muhammad Toyeeb carried out the statistical analysis.

Conflict of Interest

The authors declare no conflict of interest.

References

- Abubakar, A., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences*, 12(1), 1–10. DOI: 10.4103/jpbs.JPBS_175_19
- Adegoke, A., & Olakayode, S. (2024). Potency of butylated hydroxytoluene and optimized black pepper extract as additives on quality characteristics of stored (4 C) pork on various days. *Food Science and Biotechnology*, 33(14), 3367–3377. <https://link.springer.com/article/10.1007/s10068-024-01567-3>
- Anigboro, A. A., Akeghware, O., Avwioroko, O. J., & Tonukari, N. J. (2022). Evaluation of bioactive constituents and *In vitro* antioxidant potentials of the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata*. *Bio-Research*, 20(3), 1753–1762. DOI: 10.4314/br.v20i3.12
- Ayele, D. T., Akele, M. L., & Melese, A. T. (2022). Analysis of total phenolic contents, flavonoids, antioxidant and antibacterial activities of *Croton macrostachyus* root extracts. *BMC Chemistry*, 16(1). <https://link.springer.com/article/10.1186/s13065-022-00822-0>
- Bakir Çilesizoğlu, N., Yalçın, E., Çavuşoğlu, K., & Sipahi Kuloğlu, S. (2022). Qualitative and quantitative phytochemical screening of *Nerium oleander* L. extracts associated with toxicity profile. *Scientific Reports*, 12(1). <https://www.nature.com/articles/s41598-022-26087-0>
- Caratti, A. (2023). A New Workflow for Rationalizing Raw Data Exploration in GC× GC: Computer Vision to Unravel Diagnostic Signatures. In book of abstracts autumn school in food chemistry 2nd edition italian school in food chemistry for PhD student (pp. 26–27). Adele Papetti and Jean Daniel Coisson. <https://iris.unito.it/bitstream/2318/1952374/1/Abstract%20Book%20ASFC%202023.pdf>
- Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of experimental pharmacology*, 51–62. doi/full/10.2147/JEP.S379805
- Ghaffar, N., & Perveen, A. (2024). Solvent polarity effects on extraction yield, phenolic content, and antioxidant properties of Malvaceae family seeds: a comparative study. *New Zealand Journal of Botany*, 1–11. doi/abs/10.1080/0028825X.2024.2392705
- Godlewska, K., Pacyga, P., Szumny, A., Szymczycha-Madeja, A., Welna, M., & Michalak, I. (2022). Methods for Rapid Screening of Biologically Active Compounds Present in Plant-Based Extracts. *Molecules*, 27(20), 7094. <https://www.mdpi.com/1420-3049/27/20/7094>
- Goh, M. P. Y., Samsul, R. N. E., Mohaimin, A. W., Goh, H. P., Zaini, N. H., Kifli, N., & Ahmad, N. (2024). The Analgesic Potential of *Litsea* Species: A Systematic Review. *Molecules*, 29(9), 2079. <https://www.mdpi.com/1420-3049/29/9/2079>
- Halder, M., & Jha, S. (2023). Medicinal plants and bioactive phytochemical diversity: a fountainhead of potential drugs against human diseases. In *Medicinal plants: biodiversity, biotechnology and conservation* (pp. 39–93). Singapore: Springer Nature Singapore. https://link.springer.com/chapter/10.1007/978-981-19-9936-9_2
- Ibrahim, H., Uttu, A. J., Sallau, M. S., & Iyun, O. R. A. (2021). Gas chromatography–mass spectrometry (GC–MS) analysis of ethyl acetate root bark extract of *Strychnos innocua* (Delile). *Beni-Suef University Journal of Basic and Applied Sciences*, 10(1). <https://link.springer.com/article/10.1186/s43088-021-00156-1>
- Joubert, J. P. J. (2023). Cardiac glycosides. In *Toxicants of plant origin* (pp. 61–96). CRC Press. <https://www.taylorfrancis.com/chapters/edit/10.1201/9781003418276-3/cardiac-glycosides-joubert>
- Kato, L. S., Lelis, C. A., da Silva, B. D., Galvan, D., & Conte-Junior, C. A. (2023). Micro-and nanoencapsulation of natural phytochemicals: Challenges and recent perspectives for the food and nutraceuticals industry applications. *Advances in Food and Nutrition Research*, 104, 77–137. <https://www.sciencedirect.com/science/article/abs/pii/S1043452622000845>
- Kumar, P., Karam, N., & Choudhury, D. (2024). Assessment of the phytochemical constituents and metabolites in the medicinal plants and herbal medicine used in the treatment and management of skin diseases. In *Herbal Medicine Phytochemistry: Applications and Trends* (pp. 533–573). Cham: Springer International Publishing. https://link.springer.com/rwe/10.1007/978-3-031-43199-9_21
- Lawag, I. L., Nolden, E. S., Schaper, A. A., Lim, L. Y., & Locher, C. (2023). A modified folin-ciocalteu assay for the determination of total phenolics

- content in honey. *Applied Sciences*, 13(4), 2135. <https://www.mdpi.com/2076-3417/13/4/2135>
- Logeswaran, M. V., & Vadivel, V. (2024). Phytochemical analysis of trunk bark with branch bark, wood and stored bark of *Ficus racemosa* and comparison with other *Ficus* barks. *Trends in Phytochemical Research*, 8(1), 57-72. <https://oiccpres.com/tpr/article/view/11867>
- Ma, G., Chong, W., Qi, Y., Lu, Z., Zhang, Z., Nian, B., & Hu, Y. (2023). Can vitamin E ester derivatives be excellent alternatives of vitamin E: state of art. *Bioprocess and Biosystems Engineering*, 46(12), 1695-1709. <https://link.springer.com/article/10.1007/s00449-023-02918-w>
- Misiurek, J., Plech, T., Kaproń, B., Makuch-Kocka, A., Szultka-Młyńska, M., Buszewski, B., & Petruczynik, A. (2023). Determination of some isoquinoline alkaloids in extracts obtained from selected plants of the Ranunculaceae, Papaveraceae and Fumarioideae families by liquid chromatography and in vitro and in vivo investigations of their cytotoxic activity. *Molecules*, 28(8), 3503. <https://www.mdpi.com/1420-3049/28/8/3503>
- Muscolo, A., Mariateresa, O., Giulio, T., & Mariateresa, R. (2024). Oxidative stress: the role of antioxidant phytochemicals in the prevention and treatment of diseases. *International journal of molecular sciences*, 25(6), 3264. <https://doi.org/10.3390/ijms25063264>
- Obasi, J. C., and Obasi, A. A. (2021). Quantitative phytochemical analysis of the fungus endophytic *Aspergillus niger* isolated from *Vernonia amygdalina* leaves. *Journal of Drug Delivery and Therapeutics*, 11(4), 1–5.
- Oladeji, O. S., Adelowo, F. E., Oluyori, A. P., & Bankole, D. T. (2020). Ethnobotanical Description and Biological Activities of *Senna alata*. *Evidence-Based Complementary and Alternative Medicine*, 2020, 1–12. [doi/full/10.1155/2020/2580259](https://doi.org/10.1155/2020/2580259)
- Osunga, S., Amuka, O., Machocho, A., Getabu, A., & Onani, M. (2023). Some members of genus *Cassia* (*Senna*): Their ethnobotany, potency and prospects of drug discovery. *Indian Journal of Pharmacy & Drug Studies*, 2(2), 41-62. <https://repository.maseno.ac.ke/handle/123456789/5721>
- Rahim, N. A., Ferdosh, S., Zainuddin, N. A. N. B., & Sarker, M. Z. I. (2023). Extraction Methodologies, Phytochemical Constituents, and Biological Activities of *Senna alata* Linn: A Review. *The Natural Products Journal*, 13(2), 2-18. <https://doi.org/10.2174/2210315512666220427114719>
- Santhosh, P., Nithya, T. G., Lakshmi, S. G., Marino, G. L. S., Balavaishnavi, B., & Kamaraj, M. (2022). Assessment of phytochemicals, antioxidant, antibacterial activity, and profiling of functional molecules in a freshwater fern, *Salvinia cucullata* Roxb. *South African Journal of Botany*, 151, 275-283. <https://doi.org/10.1016/j.sajb.2022.02.030>
- Sari, K. R. P., Ikawati, Z., Danarti, R., & Hertiani, T. (2023). Micro-titer plate assay for measurement of total phenolic and total flavonoid contents in medicinal plant extracts. *Arabian Journal of Chemistry*, 16(9), 105003. <https://doi.org/10.1016/j.arabjc.2023.105003>
- Sodimu, A. I., Bichi, A. M., Bello, M. I., Salau, L. O., Magayaki, S. A., & Babale, I. I. (2024). Comparative evaluation of phytochemical composition of leaf, root and stem of copper leaf (*Acalypha wikesiana*) mull. Arg. plant in Kaduna Northern Guinea Savanna Ecological Zone, Zaria–Kaduna, Nigeria. *Journal of Research in Forestry, Wildlife and Environment*, 16(2), 126-129. <https://www.ajol.info/index.php/jrfwe/article/view/275313>
- Thamer, F. H., & Thamer, N. (2023). Gas chromatography – Mass spectrometry (GC-MS) profiling reveals newly described bioactive compounds in *Citrullus colocynthis* (L.) seeds oil extracts. *Heliyon*, 9(6), e16861–e16861. [https://www.cell.com/heliyon/fulltext/S2405-8440\(23\)04068-9](https://www.cell.com/heliyon/fulltext/S2405-8440(23)04068-9)
- Zhang, L., Xu, M., Guan, Q., Jiang, J., Sun, K., & Manirafasha, E. (2023). Determination of vegetable tannins from plants in China. *Biofuels, Bioproducts and Biorefining*, 17(3), 592-601. <https://doi.org/10.1002/bbb.2467>