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Quantitative Phytochemical and LCMS/MS Analysis of *Guiera senegalensis* Aqueous and Methanol Leaf Extracts

Hadiza Rasheed-Jada [1,3*], Peter Olatunde Ajagbonna[1], Hudu Garba Mikail[1], Motunrayo Ganiyat Akande[1], Abubakar Umar Suleiman[2], Busari A.K[2], Ishaq Abdullahi Ndagi[3].

[1]Department of Pharmacology and Toxicology,
Faculty of Veterinary Medicine,
University of Abuja,
Nigeria.

[2]National Biotechnology Development Agency (NABDA),
Abuja,
Nigeria.

[3]Celkha Biosciences LTD
Address: 11th Crescent,
Kado Estate,
FCT,
Abuja.

Email: hadizamr@gmail.com
ORCID: https://orcid.org/0000-0002-9161-5347

Abstract
Guiera senegalensis is a vital medicinal plant in African traditional medicine which has been acknowledged for its therapeutic efficacy. It is conventionally linked to numerous pharmacological actions, including anti-inflammatory, antibacterial, anticancer, cardioprotective, neuroprotective, and antiallergic characteristics. Nonetheless, a comprehensive understanding of its phytochemical makeup and bioactive components is still constrained. This work sought to examine the quantitative phytochemical composition of Guiera senegalensis leaf and also to conduct a comprehensive analysis of its bioactive components using LC-MS/MS. Aqueous and methanol leaf extracts of Guiera senegalensis were subjected to LC-MS/MS analysis to identify distinctive bioactive components. The study identified 46 phytoconstituents, comprising galloylquinic acid derivatives, phenolic acids, flavonoids, tannins, beta-carboline alkaloids, naphthopyrans, and alkaloids. The study emphasizes the health benefits of this plant and its significance as a vital resource in African traditional medicine, facilitating further research and development. Studies are currently on-going to isolate the compounds in their pure forms in order to elucidate and characterize their structures using different spectroscopic techniques.

Keywords: African traditional medicine, *Guiera senegalensis*, LC-MS/MS, Pharmacological

Introduction
African traditional medicine includes a diverse array of therapeutic practices such as herbalism, spiritualism, and divination which have been utilized for centuries to address various medical conditions (Josephine *et al*., 2019; Mokgobi, 2014; Chaitanya *et al*., 2022). The practices are fundamentally embedded in the cultural and spiritual framework of African

**Author for Correspondence*

societies, demonstrating a considerable dependence on indigenous plant species for therapeutic purposes. The continent's extensive biodiversity encompasses approximately 40,000 plant species with medicinal properties, establishing traditional medicine as a fundamental aspect of healthcare for 70-80% of the population (Mahomoodally, 2013; Mokgobi, 2014). The dependence on medicinal plants has preserved traditional knowledge and facilitated contemporary ethnopharmacological research focused on identifying bioactive compounds and their therapeutic potential (Dirar and Devkota, 2021). Despite the prevalent application of traditional African medicine, a notable deficiency persists in the scientific comprehension of the chemical composition and pharmacological characteristics of numerous medicinal plants (Okaiyeto and Oguntibeju, 2021; Fongnzossie Fedoung et al., 2023). *Guiera senegalensis* is a plant that demonstrates considerable potential in traditional African medicine. It is referred to as 'Sabara' by the Hausa people of Nigeria and 'geloki' by the Fulanis in Adamawa State, is a medicinal plant that is extensively distributed across arid regions of Africa, including Mauritania, Senegal, Mali, and Nigeria (Silva et al., 2008a; Yahaya et al., 2019). The plant has a historical application in the treatment of various ailments, such as gastrointestinal disorders, respiratory infections, rheumatism, and malaria (Anyanwu and Okoye, 2017; Hussain and Deeni, 1991). Furthermore, the leaves are thought to possess lactogenic properties, and the plant is also of cultural importance in rituals and protective charms (Silva et al., 2008a; Pirintsos et al., 2022). Recent studies have initiated the exploration of the pharmacological potential of *G. senegalensis*. The plant contains various bioactive compounds such as alkaloids, flavonoids, tannins, and naphthopyrans, which demonstrate antioxidant, anti-inflammatory, antibacterial, and anticancer properties (Fiot *et al*., 2006; Ahmed *et al*., 2022). Additionally, the galls of *G. senegalensis* exhibit significant antioxidant and anti-acetylcholinesterase properties, underscoring the plant's potential for the treatment of neurodegenerative diseases (Sombié et al., 2011; Hayat et al., 2020). Despite these promising findings, there exists a significant deficiency in comprehensive research regarding the phytochemical composition of *G. senegalensis* leaves. Although certain studies have identified specific compounds, the comprehensive range of bioactive compounds is still inadequately investigated. The existing gap in the literature restricts the advancement of standardized herbal formulations and the incorporation of *G. senegalensis* into contemporary therapeutic practices. Therefore, this research seeks to fill existing gaps through a comprehensive phytochemical analysis of *G. senegalensis* leaf, specifically to identify the bioactive compounds in the methanol and aqueous extracts, thereby providing a scientific foundation for the traditional use of *G. senegalensis* and exploring its potential for contemporary pharmacological applications.

Materials and Methods

Plant Preparation

G. senegalensis was collected from Danbatta Local Government Area, Kano State, Nigeria, and then identified by a Taxonomist at the Bioresources Development Centre, Kano, National Biotechnology Development Agency (NABDA), and a reference voucher number; BDCKN/EB/1616 was deposited in the Herbarium. The phytochemical components of *G. senegalensis* leaves were extracted by macerating the powered leaves in methanol and distilled water in order of increasing polarity for 48 hours, the extracts were then evaporated to dryness at 40 °C using a rotary evaporator and water bath (Wasihun *et al*., 2023).

Quantification of Phytochemicals

i- Total Alkaloids Content: This was calculated as mg atropine equivalent/g of each extract (Das *et al*., 2018). The formula for the calculations is as follows:

Total alkaloid content (mg/g) = (C × V) / M

Where: T is the total content of alkaloids in mg/g plant extract, C is the concentration of atropine from the calibration curve in µg/mL, V is the volume of extract in mL and M is the weight of the water extract of the plant in g.

ii- Total Phenolic Content: Folin-Ciocalteu's method was used to determine the total phenolic compounds in fruit pulp extracts, using gallic acid as a standard and measuring the absorbance at 765 nm.

Total phenolic content (mg/g) = (C × V) / M

Where: C is the concentration of gallic acid from the calibration curve in µg/mL, V is the volume of the extract in mL and M is the weight of the water extract of the plant in g (Das *et al.*, 2018).

iii- Total Flavonoid Content: The flavonoid content of the dried extracts was measured using an aluminum chloride colorimetric assay with rutin as a standard and absorbance at 506 nm.

Total flavonoid content (mg/g) = (C × V) / M

Where: C is the rutin concentration from the calibration curve in µg/mL, V is the volume of the extract in mL and M is the weight of the dried extract in g (Das *et al.*, 2018; Shraim *et al.*, 2021).

LCMS/MS Analysis

This was carried out at the Mass Spectrometry Unit of Stellenbosch Bosch University's Central Analytical Facilities in South Africa. The Waters Synapt G2 quadrupole time-of-flight mass spectrometer (Milford, Massachusetts, United States) was utilized, which was connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) and an Acquity photodiode array (PDA) detector. The ionization was accomplished using an electrospray source with a cone voltage of 15 V and a capillary voltage of 2.5 kV. Subsequently, both positive and negative ionization modes were employed. Nitrogen was used as desolvation gas at a flow rate of 650 L/h, and the desolvation temperature was maintained at 275 °C.

RESULTS

Quantitative Phytochemical Analysis

The methanol extract of *G. senegalensis* leaf demonstrated superior efficacy in extracting terpenoids and flavonoids, whereas the aqueous extract produced higher concentrations of saponins and alkaloids. The phenolic compounds exhibited comparable characteristics in both extracts, with methanol demonstrating a marginal advantage.

Table 1: Quantitative Phytochemical Analysis of Methanol and Aqueous Leaf Extracts of *G. senegalensis*

Phytochemicals	Extracts	
	Methanol (%)	Aqueous (%)
Saponins	15	20
Tannins	1.6	2.72
Phenolic compounds	2.13	1.82
Alkaloids	7	8
Flavonoids	23	17.6
Terpenoids	30	10
Cardiac glycosides	0.88	0.01
Steroids	4.5	3.42

LCMS/MS Analysis of Methanol Leaf Extract

i- Positive Mode of Ionization

The LCMS/MS analysis of the methanol leaf extract of *G. senegalensis* using positive mode of ionization detected 17 peaks, the most prominent peaks have M/Z of 301.1075, 301.1079, 291.1229, 319.0456, 319.0458, 607.2922 and 287.0928 as shown in figure 1.

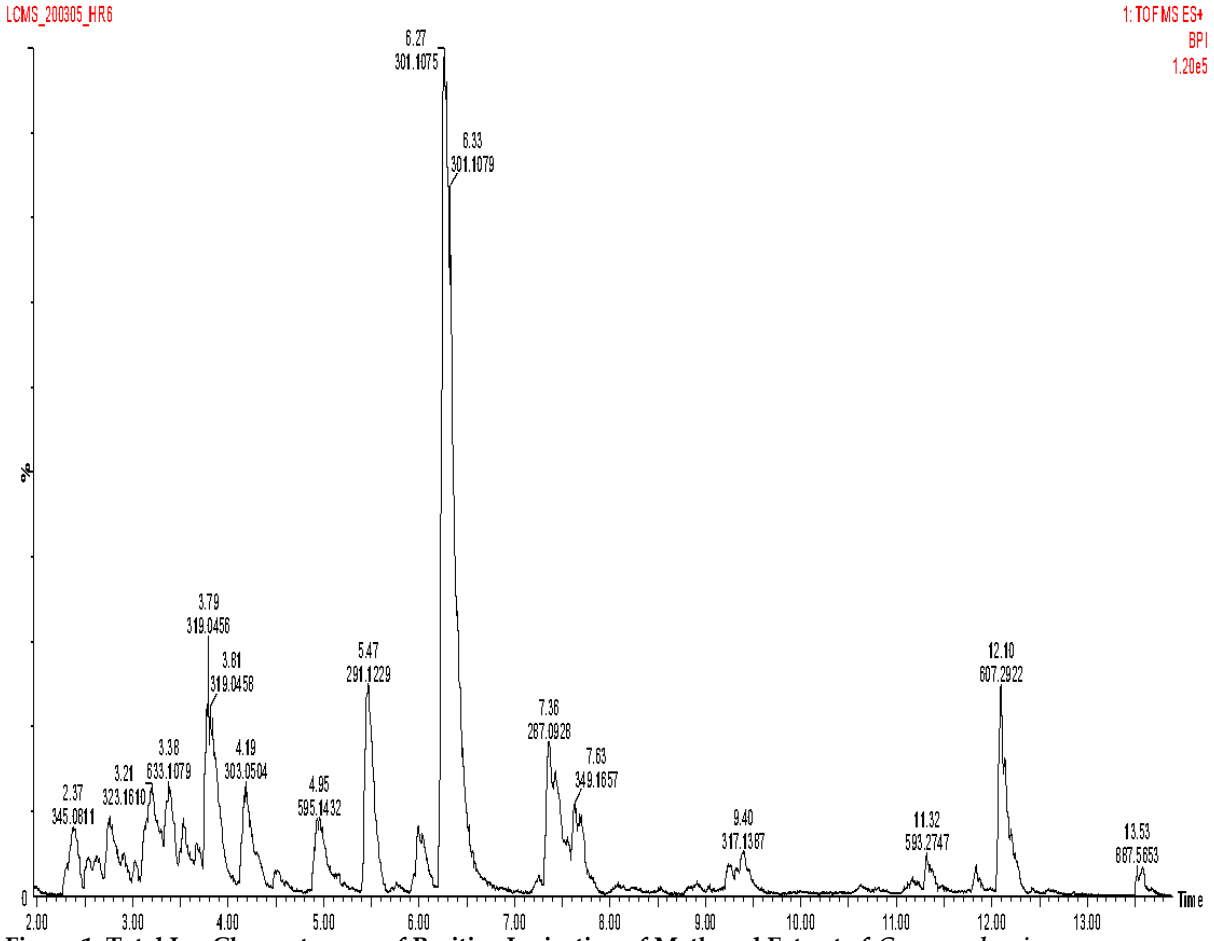

Figure 1: Total Ion Chromatogram of Positive Ionization of Methanol Extract of *G. senegalensis*

Table 2: Summary of the Total Ion Chromatogram of the Methanol Leaf Extract of *G. senegalensis* (Positive ionization)

S/N	Predicted Compound	Molecular Formula	Ontology	Molecular Weight (g/mol)	M/Z $[M+H]^+$	Retention Time (Minutes)
1	Rhodioloside;(-)-Rhodioloside;Salidroside	$C_{14}H_{20}O_7$	O-glycosyl compounds	300	301.1079	6.33
2	2-(3,4-dihydroxyphenyl)-3,5,6,7-tetrahydroxy-4H-chromen-4-one	$C_{15}H_{10}O_8$	Flavonols	318	319.0458	3.79
3	Sakuranetin	$C_{16}H_{14}O_5$	7-O-methylated flavonoids	286	287.0928	7.36
4	Myricetin 7-(6''-galloylglucoside)	$C_{28}H_{24}O_{17}$	Flavonoid-7-O-glycosides	632	633.1079	3.388
5	Argininosuccinic acid	$C_{10}H_{18}N_4O_6$	Aspartic acid derivatives	290	291.1229	5.47
6	Methyl pheophorbide a	$C_{36}H_{38}N_4O_5$	Chlorins	606	607.2922	12.10

ii- Negative Mode of Ionization

The LCMS/MS analysis of the methanol leaf extract of *G. senegalensis* using negative mode of ionization detected 24 peaks; the most prominent peaks have M/Z of 463.0860, 463.0869, 463.0862, 631.0939, 647.0887, 495.0771, 447.0902 and 447.0915 as shown in figure 2.

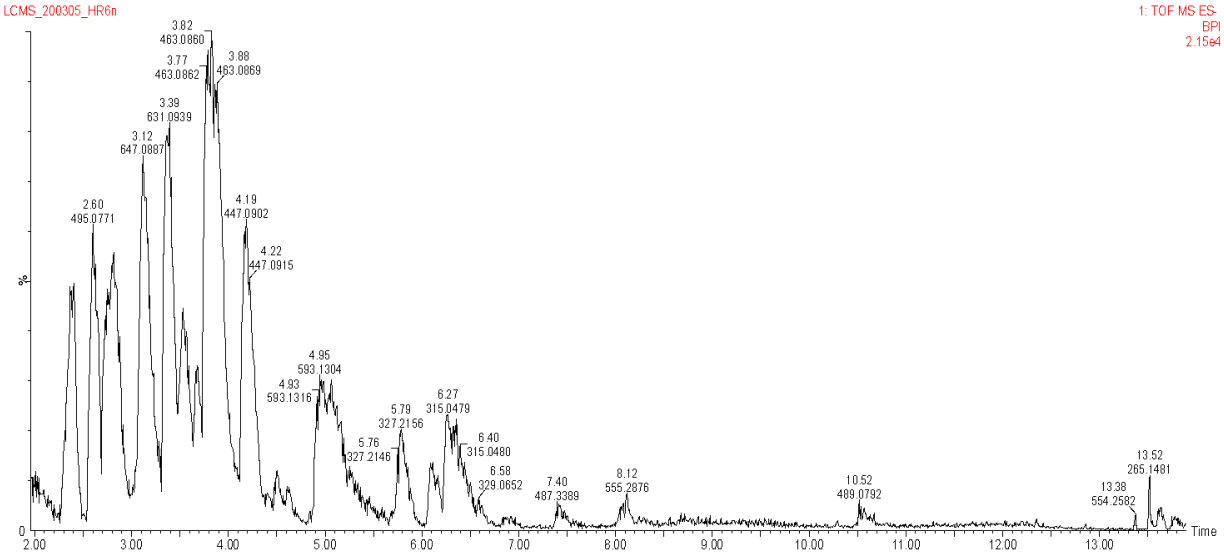

Figure 2: Total Ion Chromatogram of Negative Ionization of Methanol Extract of *G. senegalensis*

Table 3: Summary of the Total Ion Chromatogram of the Methanol Leaf Extract of *G. senegalensis* (Negative Ionization)

S/N	Predicted Compound	Molecular Formula	Ontology	Molecular Weight (g/mol)	M/Z $[M-H]^+$	Retention Time (Minutes)
1	Myricetin 7-(6''-galloylglucoside)	$C_{28}H_{24}O_{17}$	Flavonoid-7-O-glycosides	632	631.0939	3.39
2	alpha-L-Fucp-(1->2)-beta-D-Galp6S-(1->4)-D-Glcp6S	$C_{18}H_{32}O_{21}S_2$	Oligosaccharide sulfates	648	647.0887	3.12
3	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	Flavonoid-3-O-glycosides	464	463.0862	3.77

LCMS/MS Analysis of Aqueous Leaf Extract

i- Positive Mode of Ionization

The LCMS/MS analysis of the aqueous leaf extract of *G. senegalensis* using positive mode of ionization detected 28 peaks; the most prominent peaks have M/Z of 301.1086, 192.1395, 319.0457, 345.0828, 187.1236, 209.151546, 209.1542 and 680.4814 as shown in figure 3.

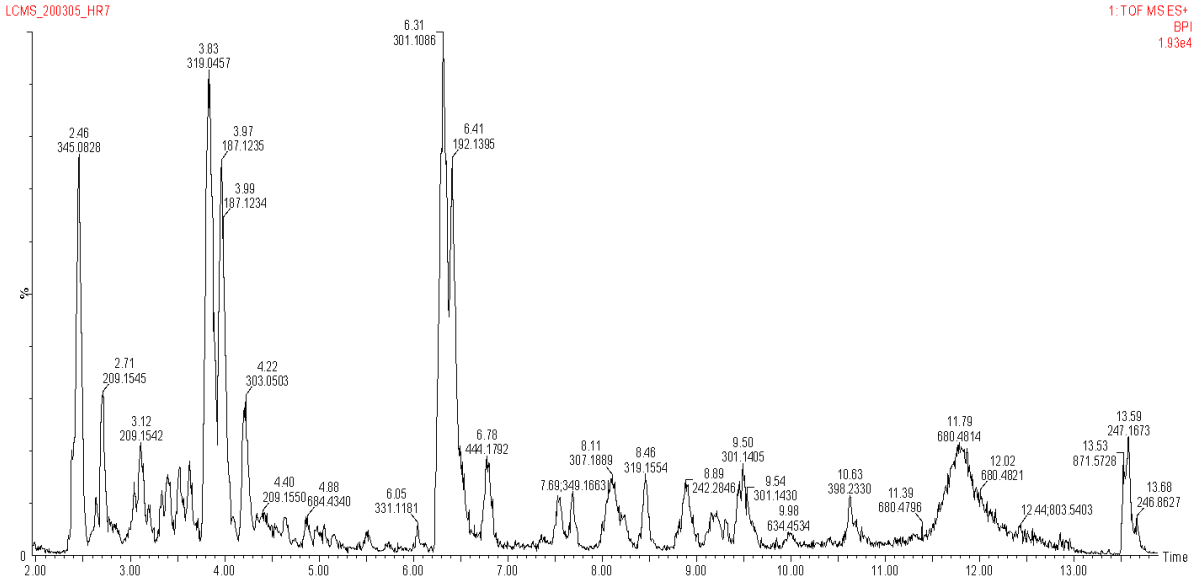

Figure 3: Total Ion Chromatogram of Positive Ionization of Methanol Extract of *G. senegalensis*

Table 4: Summary of the Total Ion Chromatogram of the Aqueous Leaf Extract of *G. senegalensis* (Positive Ionization)

S/N	Predicted Compound	Molecular Formula	Ontology	Molecular Weight (g/mol)	M/Z $[M+H]^+$	Retention Time (Minutes)
1	Rhodioloside	$C_{14}H_{20}O_7$	O-glycosyl compounds	300	301.1066	6.31
2	Phendimetrazine	$C_{12}H_{17}NO$	Phenylmorpholines	191	192.1395	6.41
3	2-(3,4-dihydroxyphenyl)-3,5,6,7-tetrahydroxy-4H-chromen-4-one	$C_{15}H_{10}O_8$	Flavonols	318	319.0457	0457
4	Nevadensin	$C_{18}H_{16}O_7$	8-O-methylated flavonoids	344	345.0822	2.48

ii- Negative Mode of Ionization

The LCMS/MS analysis of the aqueous leaf extract of *G. senegalensis* using negative mode of ionization detected 28 peaks; the most prominent peaks have M/Z 343.0656, 463.0867, 463.0870, 241.0692, 447.0912 and 183.0293 as shown in figure 4.

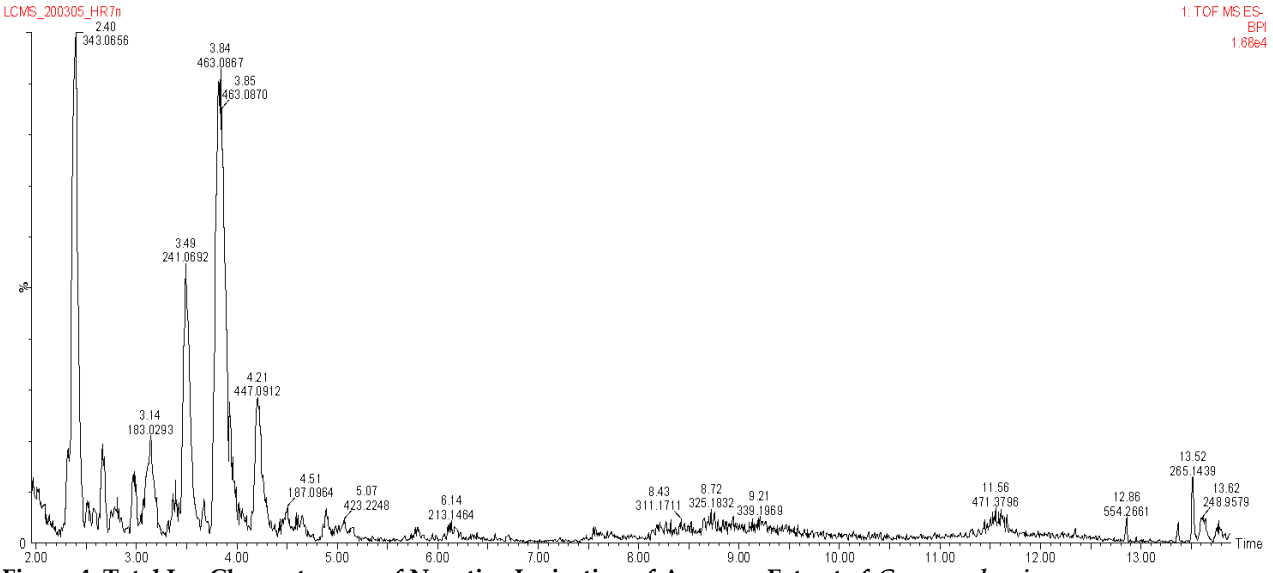

Figure 4: Total Ion Chromatogram of Negative Ionization of Aqueous Extract of *G. senegalensis*

Table 5: Summary of the Total Ion Chromatogram of the Aqueous Leaf Extract of *G. senegalensis* (Negative Ionization)

S/N	Predicted Compound	Formula	Ontology	Molecular Weight (g/mol)	M/Z [M-H]$^+$	Retention time (minutes)
1	Theogallin	$C_{14}H_{16}O_{10}$	Quinic acids and their derivatives	344	343.0658	2.40
2	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	Flavonoid-3-O-glycosides	464	463.0870	3.85
3	Thymidine	$C_{10}H_{14}N_2O_5$	Pyrimidine 2'-deoxyribonucleosides	242	241.0692	3.49

The 2D molecular structures of the compounds were sourced from the National Center for Biotechnology Information (NCBI) in the year 2024. This information was specifically retrieved from PubChem Compound on December 13, 2024, and is publicly accessible at https://pubchem.ncbi.nlm.nih.gov/compound

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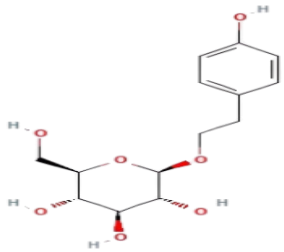
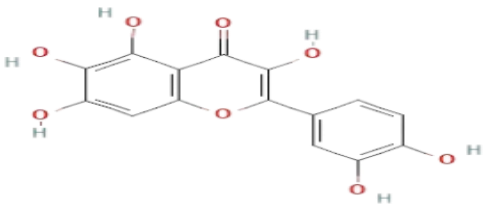
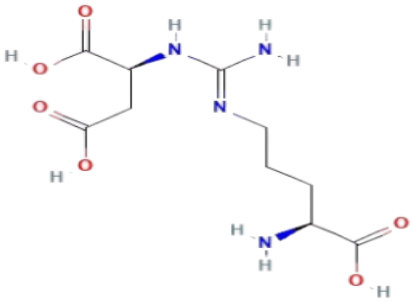
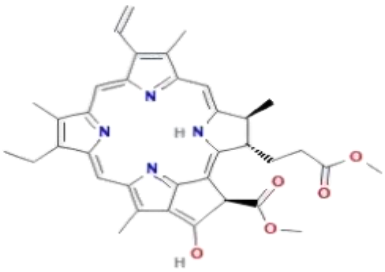
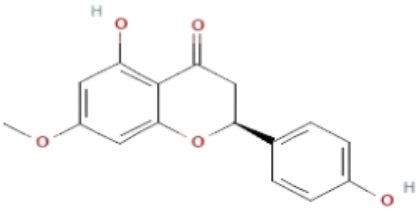
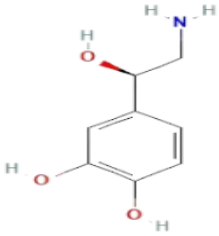
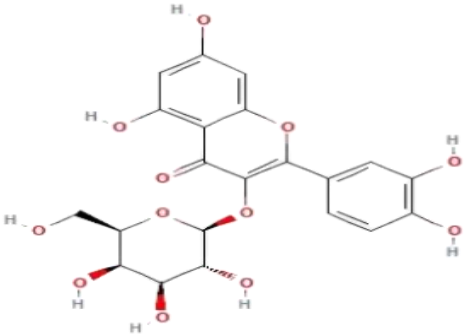
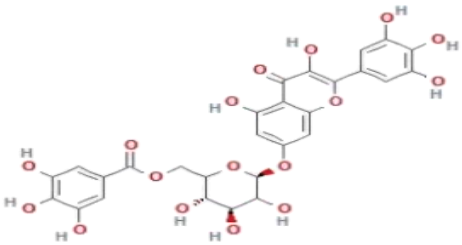
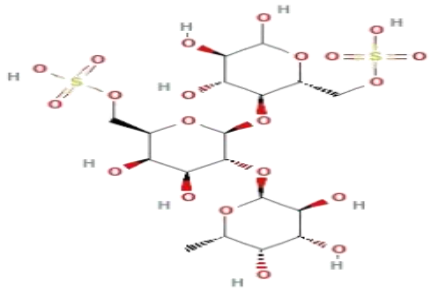
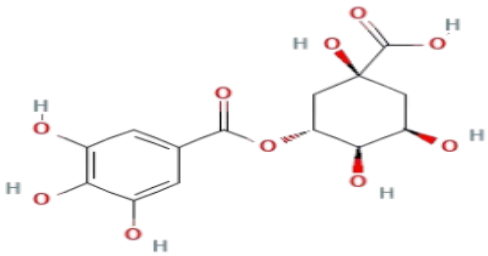
Table 6: 2D Molecular Structures of the Compounds

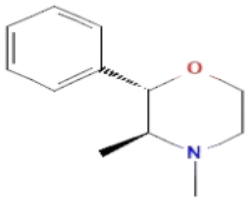
2D Structures	
	
Rhodioloside;(-) Rhodioloside; Salidroside; Tyrosol alpha-(beta-D glucopyranoside)	Argininosuccinic acid
	
2-(3,4-dihydroxyphenyl)-3,5,6,7-tetrahydroxy-4H-chromen-4-one	Methyl pheophorbide a
	
Sakuranetin	Norepinephrine
	
Quercetin 3-galactoside	Myricetin 7-(6''-galloylglucoside)



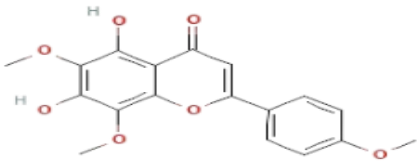
alpha-L-Fucp-(1->2)-beta-D-Galp6S-(1->4)-D-Glcp6S



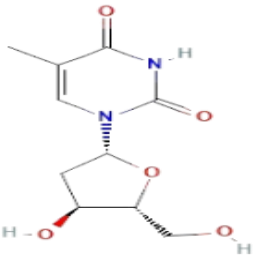
Theogallin



Phendimetrazine



Nevadensin



Thymidine

DISCUSSION

This study indicated that methanol was an exceptional solvent capable of extracting a wider array of phytoconstituents such as phenolic compounds, flavonoids, terpenoids, cardiac glycosides, and steroids, this confirmed the claim made by Kabbashi (2022). Distilled water proved to be more efficient in isolating saponins, tannins, and alkaloids, similar result was observed in the study carried out by Berlinck *et al*. (2022). The methanol extract gave a percentage yield of 84.11%, while the aqueous extract afforded 63.57%. According to Drioua *et al*., (2024), the absence of phenolic compounds and terpenoids in the aqueous extract revealed the influence of solvent polarity on the solubility and extractability of particular phytochemicals. These findings underscore the significant impact of solvent selection on the qualitative and quantitative phytochemical profiling. The compounds identified in the methanol extract include flavonoids and phenolic derivatives such as rhodioloside, (-)-rhodioloside, salidroside, tyrosol alpha-(beta-D-glucopyranoside), 2-(3,4-dihydroxyphenyl)-3,5,6,7-tetrahydroxy-4H-chromen-4-one, quercetin 3-galactoside, myricetin 7-(6''-galloylglucoside), and theogallin; these compounds have been reported to have antioxidant and anti-inflammatory properties (Reyes-Farias and Carrasco-Pozo, 2019). Terpenoids and analogous compounds such as methylpheophorbide A, sakuranetin, and nevadensin were

reported to be responsible for the antibacterial and anti-inflammatory properties of some medicinal plants (Jaiswal *et al*., 2023). Amino acid derivatives and glycosides identified include argininosuccinic acid, alpha-L-fucopyranosyl-(1->2)-beta-D-galactopyranosyl-6-sulfate-(1->4)-D-glucopyranosyl-6-sulfate, and norepinephrine could responsible for the metabolic, neuroprotective, and immunomodulatory properties of many medicinal plants (Ullah *et al*., 2020; Panche *et al*., 2016). Theogallin and quercetin 3-galactoside have been recognized for their significant antioxidant activities. The presence of norepinephrine in the leaf extract could be attributed to the neuroprotective of this plant, consistent with the plant's ethnomedicinal application in treating neurological disorders.

The phytoconstituents found in this work highlighted the ethnopharmacological significance of *G. senegalensis*, a plant long utilized in African medicine for addressing several diseases, including infections, inflammation, and neurological disorders. The identified glycosides, including alpha-L-Fucp-(1->2)-beta-D-Galp6S-(1->4)-D-Glcp6S are recognized for their immunomodulatory characteristics, which may enhance the plant's efficacy in promoting immunological health. Quercetin exhibits vasodilatory and anti-inflammatory properties, while rutin enhances capillary fortification and possesses antioxidant activity. The phytochemical composition of *G. senegalensis* indicates its potential as a source of bioactive molecules for the management of oxidative stress, inflammation, microbial infections and neurological disorders (Moreira *et al*., 2023). Subsequent research is necessary to isolate specific compounds and confirm their pharmacological effects via *in vitro* and *in vivo* models.

CONCLUSION

The present study has tentatively identified the presence of these bioactive compounds in the leaf extract of *G. senegalensis*, studies are currently on-going to isolate the compounds in their pure forms in order to elucidate and characterize their structures using different spectroscopic techniques.

Acknowledgements

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