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Phytochemical Profiling and GC-MS Analysis of Bioactive Compounds in *Guiera Senegalensis* Leaves Extract

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ABSTRACT

Medicinal plants have been used extensively as sources of a wide variety of biologically active compounds for many centuries and as crude materials or pure compounds for treating various disease conditions. The plant leaves were extracted with hexane and methanol using the Soxhlet extraction process. The Phytochemical tests were carried out on the extracts using standard procedures to identify the constituents. The result obtained revealed the presence of Flavonoids, Tannins, Phenols, and Triterpenes in Methanol and n-Hexane extracts. Saponins, Alkaloids, and Cardiac glycosides were present in Methanol but absent in n-hexane extract. Anthraquinones were absent in both extracts. The leaves of the plant have been applied in treating snakebite, stomach ache, cough, and so on. Characterization of the extracts was done using GC-MS analysis. GC-MS analysis revealed the presence of 50 compounds from which 14, 4, 15, 13, and 13 were for hexane, chloroform, ethyl acetate, butanol, and aqueous fractions, respectively. The results of this study supported the use of G. *senegalensis* in traditional medicine by demonstrating that its leaves contain phytoconstituents.

Keywords: Guiera senegalensis , Phytochemicals, GC-MS

INTRODUCTION

Plants comprise a huge number of phytoconstituents that synergistically act on different target elements of the complex cellular pathway (Kumar et al. 2013). Plant extracts have great potency and can be used for a variety of purposes. Approximately 80% of the world's population relies on traditional medicine for health care, and most therapies use plant extracts and their active compounds (Winston, 1999), suggesting that two-thirds of all plant species have medicinal value (Krishnaiah et al., 2011).

The substitution of herbal drugs with non-pharmacological substances presents a challenge as they may lack therapeutic properties, and because products derived from plants are typically variable and influenced by numerous factors, maintaining consistent product quality is crucial for the industry's survival and success (Bauer, 1998). Regulatory agencies and healthcare institutions in Africa have comparatively poor evaluations of herbal medicines compared to orthodox medicine, as opined by Falodun (2010). Thus, the evaluation of the efficacy and safety profile of medicinal plants is crucial when incorporating natural indigenous medicine into the healthcare systems of a particular country (WHO, 1996). GC-MS analysis will lead to the identification of the chemical composition of G. *senegalensis* leaves (Celestine et al., 2017). *Guiera senegalensis* (Family: Combretaceae) is a shrub of the savannah region of West and Central Africa (Anka et al., 2020). The plant generally occurs as a shrub that can grow to a height of 3 to 5 m, according to habitat. Its stem presents numerous knots that send out branches. The grey-green leaves, darker on their upper surface, display black spots on their lower surface and are slightly downy on both sides. These features lend the plant an overall silver-green colour that is conspicuous in brushland (Silva et al., 2008). In a study by Sombié (2012) G. *senegalensis* was shown to have a therapeutic effect on diarrhoea, cough, bacterial infections, human herpes, malaria, inflammations, snakebite, cancers, arterial hypertension, and diabetes. Its leaf extract is being used against dysentery, diarrhea, gastrointestinal pain and disorder, rheumatism, and fever (Sule and Muhammed, 2006). Several studies have indicated the presence of alkaloids**,** flavonoids, quercetin, catechins, saponins, tannins, amino acids, ascorbic acid, anthraquinones, and a bitter principle, elastine, in the roots and leaves of G. *senegalensis* with potential anticancer and other forms of biological activities (Jigam et al., 2011). Their functions and mechanisms of action may include the following, among others: antioxidant activity, hormonal action, stimulation of enzymes, interference with DNA replication, and antibacterial properties

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(Sule et al., 2009). The study aims to conduct phytochemical profiling and characterize bioactive compounds in the leaf extracts of G. *senegalensis*

MATERIALS AND METHODS

Sample Collection and Identification

The leaves of G. *senegalensis* were collected in November 2022 from its natural habitat in Wurno Local Government Area of Sokoto State (13.2839 ^{0}N, 5.4202 ^{0}E). The fresh leaves were identified and authenticated by Musa Magaji of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. Where a Voucher number, PCG/UDUS/Comb/0002, was obtained.

Preparation of Plant Material

The fresh leaves of G. *senegalensis* collected were washed and dried in an electrothermal oven at 37 ^{o} C. The dried leaves were coarsely powdered with an electric blender. The sample was stored in air air-tight container for further use.

Extraction

The powdered sample of G. *senegalensis* (120 g) was defatted with hexane (500 cm^3) using sohxlet apparatus at 63 -69^{o}C for 8h after which it was extracted exhaustively with methanol (500 cm^3) at 60 – 65^{o}C for 8 h; the extracts were freed from solvents using rotary evaporator at 40^{o}C to obtain Hexane (HE) and Methanol (ME) leaf extracts, respectively. Some parts of ME (18 g) were suspended in distilled water and filtered and the filtrate was successively fractionated with hexane, chloroform, ethyl acetate and butanol using liquid – liquid fractionation to obtain the different fractions coded as HE (hexane), CF (chloroform), EA (ethyl acetate), BU (butanol) and AQ (aqueous), respectively.

Phytochemical Analysis

Qualitative phytochemical tests were carried out on the crude hexane and methanol leaf extracts of G. *senegalensis* using standard procedures to identify the constituents as illustrated by Ahmad (2012). The qualitative tests conducted include the detection of alkaloids, anthraquinones, cardiac glycosides, proteins, detection of carbohydrates, tannins, saponins, flavonoids, and phenols.

The gas chromatography-mass spectrometry analysis (GC-MS)

Analysis was performed on the five fractions (HE, CF, EA, BF, and AQ) obtained from the methanol leaf extract of G. *senegalensis* using an Agilent Intuvo 9000 GC system coupled with a detector system 5977B MSD with split/splitless injection. A DB-5 MS (5% phenyldimethylsiloxane) fused silica capillary column, 30 m long, 320 µm i.d., and 0.25 µm film thickness, was used with Helium gas (99.99% purity) as the carrier gas at flow rates of 1.2 mL/min. The inlet temperature was set at 300°C, the MS Source at 230°C, and the MS Quad at 150°C. The oven temperature was programmed as follows: 50°C for 2 minutes, then increased to 250°C at a rate of 20°C per minute and held, followed by an increase to 300°C at a rate of 20°C per minute and a 3-minute hold. Data were acquired using GC-MS/Enhanced Mass Hunter Software and processed with GC-MS software, with data analysis incorporating the 2017 version of the NIST Library. One microliter of the sample extract or standard was injected in splitless mode into the GC system using the Agilent Automated Liquid Sampler (ALS) G4513A (Vijisaral et al., 2014).

Elucidation of the GC-MS chromatogram was done using the databases of NIST- National Institute of Standards and Technology and the library of WILEY. The chromatogram of the unknown compounds was compared with the spectra of already known compounds that are in the NIST and WILEY libraries. The name of the compound, peak area percentage, molecular formula, molecular weight, and structure of the sample's components were determined.

RESULTS

The extraction and fractionation yield of 120 g of G. *senegalensis* leaf afforded the following percentages, as represented in Table 1. The results of phytochemical analysis of G. *senegalensis* leaf extract are presented in Table 2. The result revealed the presence of alkaloids, phenols, flavonoids, tannins, and terpenoids in both methanol and hexane extracts. GC-MS analysis of G. *senegalensis* leaf fractions revealed the presence of several compounds. The components were identified based on peak area (%). From the results, 14, 4, 14, 13, and 13 major compounds were identified for aqueous, hexane, butanol, chloroform, and ethyl acetate fractions, respectively. The compounds are presented in Tables 3 - 7.

Table 1: Percentage Yield of G. *senegalensis* Leaf Extracts

Extracts	Weight of the extracts (g)	Yield (%)	Colour of Extracts
Hexane	6.42	5.35	Yellowish green
Methanol	22.85	19.04	Brown
Fractions			
Hexane	2.68	14.89	Yellowish green
Chloroform	2.81	15.61	Light green
Ethyl acetate	3.12	17.33	Reddish brown
Butanol	3.34	18.56	Brown
Aqueous	2.72	15.11	Light brown

Table 2: Qualitative analysis of phytochemicals present in G. *senegalensis* extracts

Phyto-constituent	Test	Extracts	
		Methanol	N-hexane
Flavonoids	Shinoda's test	+	+
	Lead acetate test	+	+
Alkaloids	Wagner's test	+	-
	Mayer's test	+	+
Tannins	Ferric chloride test	+	+
Phenols	Ferric chloride test	+	+
Saponins	Froth test	+	-
Terpenoids	Salkowski test	+	+
Cardiac glycosides	Keller Kiliani test	+	-
Anthraquinones	Modified borntrager's test	-	-

Key: (+) = Present; (-) = Absent

Table 3: GC-MS analysis of Hexane Extract from G. *senegalensis* leaves

S/No	Peak Area (%) Concentration	Compound name	Formula
1.	13.03	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
2.	1.27	2-Hydrazino-4, 6-dimethylpyrimidine	$C_6H_{10}N_4$
3.	1.09	6-Methyl-2-(3-nitrophenyl) imidazo [1, 2-a] pyridine	$C_{14}H_{11}N_3O_2$
4.	1.68	6-Chloro-4-phenyl-2-propylquinolin	$C_{18}H_{16}ClN$
5.	1.35	3-(4-nitrophenylamino) Indole	$C_{14}H_{11}N_30_2$
6.	0.98	2-Ethylacridine	$C_{15}H_{13}N$
7.	0.88	Thymol	$C_{10}H_{14}O$
8.	1.49	1, 3-diphenyl 4-[[p-(methylamino) phenyl] Amino - 2-Pyrazolin-5-one	$C_{22}H_{18}N_4O$
9.	1.84	Neophytadiene	$C_{20}H_{38}$
10.	1.25	Bis (p-phenoxyphenyl) ether	$C_{24}H_{18}O_3$
11.	14.14	n-hexadecanoic acid	$C_{16}H_{32}O_2$
12.	5.07	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
13.	3.27	Methyl stearate	$C_{19}H_{38}O_2$
14.	3.42	Tricosanoic acid	$C_{23}H_{46}O_2$

Table 4: GC-MS analysis of Chloroform Extract from G. *senegalensis* leaves

S/No	Peak Area (%) Concentration	Compound name	Formula
1.	0.12	Trichloromethane	$CHCl_3$
2.	0.13	Pentachloro ethane	C_2HCl_5
3.	3.09	Hexachloro ethane	C_2Cl_6
4.	0.03	Dichloro acetyl chloride	C_2HCl_3O

Table 5: GC-MS analysis of Ethyl Acetate Extract from G. *senegalensis* leaves

S/No	Peak Area (%) Concentration	Compound name	Formula
1.	0.19	o-Xylene	C_8H_{10}
2.	0.65	3, 4-Dihydroxy-5-methyl-dihydrofuran-2-one	$C_5H_8O_4$
3.	0.60	methoxy phenyl Oxime	$C_8H_9NO_2$
4.	2.09	2-methoxy-5-methyl Thiophene	C_6H_8OS
5.	1.03	1-tridecene	$C_{13}H_{26}$
6.	2.84	4, 4’ –methylenebis (2, 6-dimethyl) Phenol	$C_{17}H_{200}O_2$
7.	1.79	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
8.	2.13	methyl. beta.-D-glucopyranose	$C_7H_{14}O_6$
9.	0.30	4, 5- dihydro- 3H-1, 2, 4-triazole-3-thione	$C_2H_3N_3S$
10.	0.64	2-oxo- Octadecanoic acid, methyl ester	$C_{19}H_{36}O_3$
11.	0.25	2, 3-dihydro-3, 3-dimethyl-4-nitro- Benzofuran-2-one	$C_{10}H_9O_4$
12.	0.19	Bis (m-phenoxyphenyl) ether	$C_{24}H_{18}O_3$
13.	0.42	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$
14.	13.98	2-(1, 3-benzodioxol-5-yl) methoxy-5-Benzofuranpropanol	$C_{19}H_{18}O_6$
15.	13.17	6-(4-ethylphenyl) - [1, 2-b] 1,2,4-triazine -2-phenyl imidazo	$C_{18}H_{14}N_4$

Table 6: GC-MS analysis of Butanol Extract from G. *senegalensis* leaves

S/No	Peak Area (%) Concentration	Compound name	Formula
1.	1.02	4-methylpentyl Cyclohexane	$C_{12}H_{24}$
2.	1.99	2-methyl-1-methylmannopyranoside	$C_7H_{14}O_6$
3.	0.74	1, 1’-(2-ethyl-1, 3-propanediyl) bis- Cyclohexane	$C_{17}H_{32}$
4.	0.75	Cyclohexyl Cyclooctane	$C_{14}H_{26}$
5.	1.00	1-Tetradecene	$C_{14}H_{28}$
6.	7.03	1, 6-anhydro-.beta.-D-Glucopyranose	$C_6H_{10}O_5$
7.	2.02	Cetene	$C_{16}H_{32}$
8.	0.71	6-deoxy methyl.alpha.-L-Galacopyranoside	$C_7H_{14}O_5$
9.	1.73	1-Octadecene	$C_{18}H_{36}$
10.	1.12	Tricosanoic acid	$C_{23}H_{46}O_2$
11.	0.67	1-methylethyl – Cyclohexane	C_9H_{18}
12.	1.94	1H-Indole-3-carboximidamide	$C_9H_9N_3$
13.	0.68	1, 3-butadienyl 2-(3-methyl- 1, 3, 3-trimethyl-Cyclohexanol	$C_{14}H_{24}O$

Table 7: GC-MS analysis of Aqueous Extract from G. *senegalensis* leaves

S/NO	Peak Area (%)	Compound name	Formula
	Concentration		
1.	1.22	1, 1-(1, 3-propanediyl) bis Cyclohexane	$C_{15}H_{28}$
2.	1.04	1, 1'-(2-ethyl-1, 3-propanediyl) bis- Cyclohexane	$C_{17}H3_2$
3.	1.30	1-Tetradecene	$C_{14}H_{28}$
4.	5.31	Dodecanoic acid, methyl ester	$_{13}H_{26}O$
5.	0.92	Dodecane	$C_{12}H_{26}$
6.	1.29	2-propyl-, (S) – Piperidine	$C_8H_{17}N$
7.	5.48	4-Cyclopropylmethylbenzonitrile	$C_{12}H_{14}N$
8.	2.57	Cetene	$C_{16}H_{32}$
9.	3.09	Methyl tetradecanoate	$C_{15}H_{30}O_2$
10.	2.10	1-Octadecene	$C_{18}H_{36}$
11.	0.64	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
12.	1.29	Tricosanoic acid	$C_{23}H_{46}O_2$
13.	0.75	E-15-heptadecenal	$C_{17}H_{32}O$

DISCUSSION

The methanol extract at 60-65 ^{0}C has a greater yield (19.04 %) than extraction with hexane (5.35 %) at 63-69 ^{0}C (Table 1). The efficiency of methanol as a solvent used for the extraction of the plant might be due to it being an organic polar solvent. The methanol polarity allows it to have strong interactions with polar substances (Lokeswari et al., 2011). The colour of the extract observed was brown in methanol and yellowish green in the hexane extract. The colour of fractions observed was yellowish green in hexane, light green in chloroform, reddish brown in ethyl acetate, brown in butanol, and light brown in aqueous fraction.

Phytochemical screening of *G. senegalensis* crude extract revealed the presence of secondary metabolites. The result obtained revealed the presence of flavonoids, tannins, phenols, and triterpenes in methanol and n-hexane extracts. Saponins, alkaloids, and cardiac glycosides were present in the methanol but absent in the n-hexane extract. Anthraquinones were absent in both extracts. This result is similar to the findings noticed by Eltoum et al. (2020), who identified the presence of flavonoids in *G. senegalensis* methanol extract. Plant potential antioxidant activity is proportional to the amount of cell-reinforcing chemicals present, such as phenolic compounds that are capable of catalyzing free radical scavenging (Almulaiky et al, 2020). Alkaloids have pharmacological activities, such as increasing blood pressure, reducing pain, and fighting microbial infections. Alkaloids possess antibacterial (Yan et al., 2021), anticancer (Mondal et al., 2019), and anti-inflammatory (Bai et al., 2021). In plants, alkaloids are toxic substances against insects or plant-eating animals because of their bitter taste. Phenolic compounds play an important role in electron transport in photosynthesis, cytokinin activity, and growth promoters. Saponins have antipathogenic and antimicrobial activity. Saponins increase seed germination and inhibit tumor cells in plants and animals.

Plant tannins are polyphenols widely found in terrestrial plants and some marine plants (phloroglucinol). Tannins have been reported to function as antibacterial and have antitumor and antiviral activity (Kumari et al., 2012). Tong et al. (2022) reported that tannins inhibit the growth of pathogenic bacteria and fungi, have antibacterial, antioxidant, and diuretic properties, and act as an insecticide. The compounds present in the fractions of *G. senegalensis* were identified by GC-MS analysis. Fifty compounds, which were the major components, were identified. The active principles with their molecular formula and concentration (% Area) in the methanol fractions of *G. senegalensis* leaves.

The result revealed that Dodecanoic acid, methyl ester (5.31%), 4-Cyclopropylmethylbenzonitrile (5.48%), and Methyl tetradecanoate (3.09%) in the aqueous fraction are the major compounds. On the other hand, Hexadecanoic acid, methyl ester (13.03%), n-hexadecanoic acid (14.14%), 10-Octadecenoic acid, methyl ester (5.07%), Methyl stearate (3.27%), Tricosanoic acid (3.42%) were prevalent in the hexane fraction. In addition, 2-methyl-1-methylmannopyranoside (1.99%), 1H-Indole-3-carboximidamide (1.94%), 1, 6-anhydro-β-D-glucopyranose (7.03%) were the major compounds in Butanol Fraction. Hexachloro-ethane (3.09) in the chloroform fraction. 2-Methoxy-5-methyl thiophene

(2.09%), 4, 4' –methylenebis (2, 6-dimethyl) Phenol (2.84%), Methyl.beta.-D-glucopyranose(2.13%), 2-(1,3-benzodioxol-5-yl)methoxy-5-Benzofuranpropanol (13.98%), 6-(4-ethylphenyl)-phenyl Imidazo([1, 2-b] 1,2,4-triazine (13.17%) and 2-Methyl-7-phenylindole(6.68%) in ethyl acetate fraction were found as the major compounds. Among the identified phytochemicals, n-hexadecanoic acid has the property of antioxidant activity as reported by earlier studies (Lalitharani et al., 2009; Maruthupandian and Mohan, 2011).
Beta-D-Glucopyranose, 1, 6-anhydro- shows antibacterial and antioxidant activity (Igwe et al., 2013).

Furthermore, n-Hexadecanoic acid was reported to have antioxidant, hypocholesterolemic, nematicidal, pesticidal, hemolytic, 5-alpha reductase inhibitor, anti-androgenic, hemolytic (Kumar et al., 2010), and anti-inflammatory activity (Aparna et al., 2013).
Hexadecanoic acid, methyl ester possesses some biological activity such as antioxidants, hypocholesterolemic, nematicide and pesticide (Sheela et al., 2013).
Febri et al. (2016) reported the antibacterial activity of Dodecanoic acid against Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium, and Escherichia coli at a concentration of 5%. This is consistent with a similar study conducted by Moigradean et al. (2013), which indicated the presence of Hexadecanoic acid which is also known as Palmitic acid, which is also present in walnut and coconut oil, and has anti-bacterial property (Yff et al., 2002). Bis (p-phenoxy phenyl) ether was found to possess anti-androgenic activity (Abeer et al., 2017). Studies have shown that 2-(1, 3-benzodioxol-5-yl) methoxy-5-benzofuranpropanol has good antioxidant activity (Rhinde et al., 2010).
6-(4-ethylphenyl)-2-phenylimidazo [1, 2-b] [1, 2, 4] triazine possesses antituberculosis (Gong et al., 2015), antioxidant, antiviral, antitumor (Hamama et al., 2016), antifungal (Lafleur et al., 2011), and antiparasitic (Thompson et al., 2020) activities. The prevailing compounds found in the leaf of G. *senegalensis* fractions are lipid components. Fatty acids and derivatives in plant extracts have been widely reported to inhibit cancer cell proliferation by induction of apoptosis (Alasmari et al., 2015; Leyva-Peralta et al.,2015).A clear example of this was the extract from Euphorbia kansui, which contained a high amount of octadecenoic acid, methyl ester as the major compound that triggered apoptosis in a human gastric cancer cell line (Ismail et al., 2019). All the compounds have previously been reported from several other plant species.

CONCLUSION

Phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, Anthraquinones, phenols, Saponins, and terpenoids. The identified metabolites are believed to be responsible for the plant's therapeutic activity. This study further validates the use of GC-MS to reveal the composition and concentration distribution of compounds that are identifiable when analyzing unidentified components of plant origin. All the major compounds from the different fractions are biologically active molecules. Considering these findings, the traditional uses of the leaves appeared to be justified.

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Conflict of interest

The authors declare no conflict of interest.

Author's contributions

A Uba, U Z Faruk, and T Izuagie conceptualized and designed the study. SM Zayyan carried out the research work and acquired the data. All the authors analyzed the data. Finally, all the authors edited the manuscript and approved the final version for submission.

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