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Phytochemical Characterization by Gc-Ms Analysis of the Ethyl Acetate Leaf Extract of Combretum Micranthum G.Don Harvested in the Kita Region of Mali

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ABSTRACT

Combretum micranthum G.Don (Combretaceae) is a medicinal plant widely known and used in Africa to treat various ailments, including diabetes, fever, cough, bronchitis, diarrhea, pain, malaria, and liver disorders, among others.

The objective of this study was to characterize the plant, harvested in Mali, to identify the presence of biologically active phytochemicals in ethyl acetate extracts. This study was conducted using gas chromatography-mass spectrometry (GC-MS), and the mass spectra of the compounds identified in the extract were compared to the National Institute of Standards and Technology (NIST) database. In this study, ethyl acetate extracts of *Combretum micranthum* G.Don were analyzed using standard qualitative tests to detect the presence of steroids, alkaloids, tannins, flavonoids, terpenoids, and cardiotonic glycosides. However, it should be noted that anthraquinones were not found in our extracts. This study was then further investigated by analyzing the bioactive compounds present in the plant's ethyl acetate extracts using GC-MS. The analysis revealed the presence of approximately one hundred different compounds in the *Combretum micranthum* G.Don ethyl acetate extracts, including alkaloids, terpenoids, steroids, saturated and unsaturated fatty acids, aromatic and aliphatic hydrocarbons, and polyphenolic compounds. The results confirm the presence of compounds with therapeutic potential in the ethyl acetate extracts of *Combretum micranthum* G.Don, mainly steroids, flavonoids and terpenoids.

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INTRODUCTION

Previous research on *C. micranthum* was conducted mainly by West African scientists in the 20th century and focused on leaf tissue chemistry, as we did in this study, due to the traditional use of the leaves. The compounds identified in these studies were essentially limited to fatty acids, flavones, and triterpenes, as well as a few simple alkaloids and sugar alcohols. In one of the earliest phytochemical investigations, Paris, in 1942, was able to isolate a compound, which was probably catechin, and polymerized catechin, but did not specify the structures due to a lack of material (Paris, 1942). In 1962, Jentzsch et al. reported the discovery of vitexin and, very likely, orientin, as well as the alkaloids choline and betaine, and the phenolic acids oxalic acid, malic acid, and gallic acid (Jentzsch et al., 1962). Dr. Emmanuel Bassene has conducted the most thorough work to date on the constituents of *C. micranthum*, allowing both the confirmation of some previously identified compounds and the refutation of others. In 1981, Bassene et al. reported the identification of sugar alcohols, m-inositol and sorbitol, as active principles of leaf extracts with diuretic properties (Bassene et al., 1981). He also isolated at least a dozen fatty acids, the three main ones

being palmitic acid, oleic acid, and linoleic acid (Bassene et al., 1986). He also confirmed the presence of hydroxystachydrine, stachydrine, and choline, while contesting the evidence for the presence of the alkaloid betaine in the Kinkeliba leaf extract (Bassene et al., 1986). In 1987, Bassene et al. published the isolation of eight flavonoids, four of which were identified as vitexin, isovitexin, orientin, and homoorientin, and four others in concentrations too low to obtain structural information (Bassene et al., 1985; Bassene et al., 1987). It is likely that Bassene isolated the O-galloyl-C-glycosylflavones; these compounds were not formally elucidated until 2002 by Latte et al., who isolated them from Pelargonium reniforme (Latte et al., 2002). Bassene completed his exploration of *C. micranthum* by identifying several aliphatic alcohols, two types of triterpenic alcohols (α-amyrin and lupeol), and one sterol (β-sitosterol) (Bassene et al., 1989). Finally, D'Agostino et al. confirmed the presence of alkaloids, phenolic acids, sugar alcohols, and flavone-C-glycosides, as well as identifying and quantifying two new flavonol glycosides, myricetin-3-O-glucoside and myricetin-3-O-rutinoside (D'Agostino et al., 1990). The present study of *C. micranthum* leaves was initiated to characterize medicinally active compounds using bioactivity-guided fractionation. The following phytochemical research, under bioactive guidance, focuses on the ethyl acetate fraction of the crude alcoholic extract, and has led to the detection of various compounds, all of which have been previously reported in the literature.

MATERIALS AND METHODS

Materials

Plant Materials

The sample used in this study came from the Kita region of Mali. It was harvested in April 2022. After being sorted and cleaned, the leaves were left to air dry in the dark. They were then ground using an electric grinder. The resulting powder was then sieved (using an electric sieve (Retsch-AS 200)) and stored in glass jars away from light to prevent deterioration before use. **Preliminary Phytochemical Analysis**

Chemical tests were performed on ethyl acetate extracts of plants and/or on powdered samples, according to standard procedures for identifying active constituents (Harborne J.B., 1973; Sarker S.D. et al., 2005; Bekro et al., 2007; Kokate C. et al., 2009; Badiaga, 2012; Zitouni, 2017).

Alkaloid Test

10 mL of alcoholic extract were stirred with 5 mL of 1% HCl in a water bath. Mayer's reagent (1.35 g mercuric chloride in 60 mL of water + 5 g potassium iodide in 10 mL of water) and Wagner's reagent (1.27 g iodine and 2 g potassium iodide in 100 mL of water) were added. The formation of white and reddish-brown precipitates, respectively, was considered evidence of the presence of alkaloids.

Flavonoid Test

(a) Lead acetate test: 1 mL of a 10% lead acetate solution was added to 5 mL of alcoholic extract. The formation of a yellowish-white precipitate was considered a positive result for the presence of flavonoids.

(b) Sodium hydroxide (NaOH) test: 5 ml of extract were treated with an aqueous solution of sodium hydroxide and hydrochloric acid (HCl), and the appearance of an orange-yellow color was observed.

Anthocyanin detection

To the 5% infusion exhibiting a more or less dark color, add an acid (2 ml of H_2SO_4) then a base (10 ml of 50% NH_4OH or 10% NaOH). If the color intensifies upon acidification and then turns blue-violet in a basic medium, the presence of anthocyanins can be concluded.

Steroid detection

(a) Liebermann-Burchard test: 3 ml of extract were treated with chloroform, acetic anhydride, and a few drops of sulfuric acid were added. The appearance of a dark pink or red color indicates the presence of steroids.

(b) Sulfuric acid (H_2SO_4) test: The appearance of a greenish color was considered an indicator of the presence of steroids when 2 ml of organic extract was treated with sulfuric and acetic acids.

Tannin test

10 mg of plant material were dissolved in 10 ml of distilled water and filtered. The filtrate (3 ml) was then mixed with 3 ml of a 5% w/v $FeCl_3$ solution. The formation of a dark green or blue-black precipitate was considered an indicator of the presence of tannins.

Anthraquinone Tests

Borntrager Test: 3 mL of alcoholic extract were shaken with 3 mL of benzene, filtered, and then 5 mL of a 10% ammonia solution were added to the filtrate. After shaking, the appearance of a pink, red, or purple color in the ammonia (lower) phase indicates the presence of free anthraquinones.

Terpenoid Test

2 mL of alcoholic extract were dissolved in 2 mL of chloroform and evaporated to dryness. 2 mL of concentrated sulfuric acid were then added, and the mixture was heated for approximately 2 minutes. A grayish color was considered an indicator of the presence of terpenoids.

Cardiotonic Glycosides

Test Keller-Kiliani Test: Alcoholic extract (2 ml) + 1 ml glacial acetic acid + $FeCl_3$ + concentrated H_2SO_4. The appearance of a blue-green color indicates the presence of cardiotonic glycosides. Extract Preparation: 120 coarsely ground, shade-dried seeds were defatted separately with hexane for 24 hours and then left to dry at room temperature. The defatted plant material was extracted with 80% methanol in a Soxhlet apparatus until complete exhaustion. The alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40°C to give a dark brown residue

designated as the crude extract. The alcoholic extract was then analyzed by GC-MS.

Sugar and Holoside

Tests The 10% aqueous decoction (5 ml) was evaporated to dryness, then 2 to 3 drops of concentrated H_2SO_4 were added to the residue, followed after 5 minutes by 3 to 5 drops of alcohol saturated with thymol. The development of a red color indicates the presence of sugars and holosides.

Saponoside Tests

The solution to be analyzed was a 1% decoction. In an Erlenmeyer flask, distilled water (100 ml) was brought to a boil, and 1 g of plant drug powder was added. A moderate boil was maintained for 15 minutes. The solution was filtered and, after cooling, adjusted to 100 ml. In a series of 10 test tubes numbered 1 to 10, successively (1 to 10 ml) of the decoction was added.

A 1% solution was used. The volume of each tube was adjusted to 10 mL with distilled water. Each tube was shaken lengthwise for 15 seconds and then left to stand for 15 minutes. The foam height was then measured. The foam index (FI) was calculated from the tube in which the foam height was 1 cm (N).

Mucilage detection

Add 5 mL of absolute ethanol to 1 mL of the 10% decoction. The formation of a flocculent precipitate upon mixing indicates the presence of mucilage.

Coumarin detection

Evaporate the 5 mL of ether extract obtained after a 24-hour maceration until dry, then dissolve the residue in 2 mL of hot water. Divide the solution between two test tubes. Add 25% ammonia (0.5 ml) to one of the tubes and observe fluorescence under UV light at 366 nm. Intense fluorescence in the tube where ammonia was added indicates the presence of coumarin.

Methods

Decoction

100 g of *Combretum micranthum* G.Don powder was added to 1 liter of water. The mixture was boiled for 15 minutes at 100°C in a stainless-steel cup. After cooling, it was filtered through cheesecloth, and the filtrate was concentrated using a Rotavapor at 55°C and then freeze-dried (Hawa, 2019).

Infusion

This method involves infusing 10 g of *Combretum micranthum* G.Don powder in 100 ml of distilled water, initially brought to a boil, for 30 minutes. The infusion was then left in the dark until cool. The resulting filtrate was centrifuged at 4000 rpm for 20 minutes and stored at 4°C until use (Hawa, 2019).

Methanolic extraction

A 200 mg mass of *Combretum micranthum* G.Don leaf powder was macerated with 2 L of methanol for 24 hours using a stirrer (Fathiazad et al., 2011). This operation was repeated successively for three days to extract as much of the phytochemical compounds as possible from the powders. After vacuum filtration (using a pump) through Whatman filter paper, the resulting hydro-alcoholic phase was evaporated to dryness under reduced pressure in a rotary evaporator at 50°C to remove the methanol.

Liquid-liquid fractionation of ethyl acetate extract

The methanolic dry extracts (EMeOH) from the previous operation were used for fractionation after 72 hours of drying. A mass of 150 mg of dry residue was weighed and dissolved in 1.5 L of slightly heated distilled water, then filtered after cooling and evaporated to dryness. The residual aqueous phase was successively reconstituted with ethyl acetate, n-butanol, and dichloromethane for 24 hours using the same volume. The solutions were filtered and evaporated to dryness to obtain the fractions (FEtOAc) and stored at 4°C in a refrigerator before use (Bekkara et al., 1998).

Isolation and Purification

A quantity of 2 g of dry ethyl acetate extract was prepared for silica gel separation by dissolving it in cyclohexane and adhering it to dry silica. The sample was then subjected to a powder rotation. The silica gel column was eluted with a gradient starting at 100% cyclohexane and then progressively with a cyclohexane/dichloromethane mixture at different volumes. Thin-layer chromatography (TLC) plates were used to monitor the eluted fractions, and a total of 10 initial fractions were obtained by grouping test tubes according to their Rf values. Some fractions showed single spots along the TLC plate, while others showed multiple spots.

Gas Chromatography/Mass Spectrometry (GC-MS)

GC-MS analysis was performed using an Agilent 7890A instrument equipped with a capillary column (Agilent 5975C intert MSD). The temperature started at 50°C, was held for 5 minutes, and then gradually increased to 200°C and subsequently to 300°C, with each temperature held for 10 minutes. Helium was used as the carrier gas, and injection was performed in split mode with a 1:30 ratio. Mass spectra were acquired in the m/z range of 30–500 with an ionization voltage of 70 eV. The Kovats retention index was calculated using standard hydrocarbons as a reference. Compounds were identified by analyzing their retention times and fragmentation profiles using mass spectra generated by GC-MS. The active components of the extract were further investigated by comparing their retention indices, peak area percentages, and mass spectral fragmentation profiles with reference data from the National Institute of Standards and Technology (NIST) digital library. This comparison confirmed the names, molecular weights, chemical formulas, structures, and bioactivities of the identified compounds (Sumner et al., 2007).

RESULTS

The results of the qualitative analysis of subfractions of ethyl acetate extract from whole leaves of *Combretum micranthum* G.Don are presented in Table 1.

Table 1: Phytochemical Screening of *C. micranthum* Ethyl Acetate Extract

⎫Abundant presence +++ ⎫Presence of compound ++ ⎫Weak reaction + ⎫Absence of compound –

Phytochemical compounds	Ethyl acetate extracts of *C. micranthum*
Alkaloids	++
Tannins	+++
Flavonoids	++
Anthocyanins	++
Anthraquinones	-
Steroids	++
Saponins	++
Terpenoids	++
Susanoids/Holosides	+
Heterosides	+
Mucilage	+
Coumarins	+
Foam Index	**111**

This analysis revealed the presence of tannins, flavonoids, alkaloids, terpenoids, saponins, anthocyanins, and steroids, as well as the absence of anthraquinone, in our ethyl acetate leaf extract of *Combretum micranthum G.Don*.

The compounds present in our ethyl acetate leaf extracts of *Combretum micranthum* G.Don were identified by GC-MS. The active compounds, along with their retention time (RT), molecular weight, CAS, percentage of surface area, and peak quality, are presented in Table 2.

Table 2: Characterization of the ethyl acetate leaf extract of *Combretum micranthum* G.Don by GC-MS analysis

Number of Peaks	Retention Time	Percentage of Surface	Compounds	Height	CAS	Quality
1	9,790	1,51	2-Propenoic acid, pentadecyl ester	122812	043080-23-5	91
2	11,694	1,84	Hexadecanoic acid, methyl ester	113682	000112-39-0	99
3	11,981	13,31	n-Hexadecanoic acid	102726	000057-10-3	99
4	12,214	3,81	Behenic alcohol	156095	000661-19-8	94
5	13,085	1,49	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	130794	000301-00-8	99
6	13,166	3,31	Phytol	133806	000150-86-7	91
7	13,372	6,25	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	119801	000463-40-1	99
8	13,740	2,71	Octadecyl trifluoroacetate	180503	079392-43-1	94
9	15,239	1,74	Cyclotetracosane	163070	000297-03-0	99
10	16,137	3,08	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	158684	023470-00-0	58
11	17,627	1,57	Octadecanoic acid, 2,3-dihydroxypropyl ester	176318	000123-94-4	64
12	18,417	17,98	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	198715	000111-02-4	99

13	21,137	5,44	Vitamin E	203745	000059-02-9	99
14	22,860	5,92	Stigmasterol	199250	000083-48-7	99
15	23,668	14,25	gamma-Sitosterol	199879	000083-47-6	99
16	24,628	2,95	13,27-Cycloursan-3-one	202287	1000196-72-7	53
17	24,862	2,86	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	202807	000469-38-5	56
18	25,068	2,03	Cholest-7-en-3-one, 4,4-dimethyl-	199260	013490-56-7	56
19	25,643	1,84	9,10-Methanoanthracen-11-ol, 9,10-dihydro-9,10,11-trimethyl-	98079	126615-74-5	38
20	25,885	6,12	Stigmast-4-en-3-one	199256	001058-61-3	95
21	10,643	3,40	5-Octadecene,(E)-	99558	007206-21-5	95
22	11,783	2,42	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	118306	082304-66-3	99
23	12,035	2,01	18-Norabietane	107437	1000293-16-6	64
24	12,223	6,65	Cycloeicosane	121341	000296-56-0	97
25	12,923	1,87	1-Octadecene	99556	000112-88-9	97
26	13,175	10,46	Phytol	133806	000150-86-7	91
27	13,740	5,64	1-Docosene	142951	001599-67-3	96
28	14,988	2,59	1,3-Dimethyl-5-n-decylcyclohexane	99572	086710-36-3	43
29	15,239	2,90	Cyclotetracosane	163070	000297-03-0	99
30	18,094	9,33	13-Docosenamide, (Z)-	163527	000112-84-5	95
31	18,417	10,77	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	198715	000111-02-4	99
32	18,848	3,13	Bis(2'-hydroxy-3'-isopropylisobutyrophenonato)beryllium(ii)	201124	025978-22-7	47
33	19,198	2,22	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	202831	054159-46-5	87
34	21,137	3,10	Vitamin E	203745	000059-02-9	99
35	23,668	3,73	Beta-Sitosterol	199877	000083-46-5	90
36	24,646	2,83	Lup-20(29)-en-3-one	202284	001617-70-5	70
37	24,880	7,21	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	202807	000469-38-5	92
38	25,077	5,56	Cholest-7-en-3-one, 4,4-dimethyl-	199260	013490-56-7	64

39	25,652	1,83	Formic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-a]pyrimidin-5-yl)-, methyl ester	64496	074772-16-0	25
40	25,921	12,34	Stigmast-4-en-3-one	199256	001058-61-3	95
41	10,652	5,25	1-Octadecene	99556	000112-88-9	95
42	11,083	1,67	2-Pentadecanone, 6,10,14-trimethyl-	112036	000502-69-2	91
43	11,702	5,69	Hexadecanoic acid, methyl ester	113690	000112-39-0	98
44	11,783	1,98	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	118306	082304-66-3	99
45	12,043	4,67	3b,4,5,6,7,7a,9,10,11,12-Decahydrobenzo[b]fluoranthene	107464	1000080-20-7	64
46	12,223	7,85	1-Nonadecene	110437	018435-45-5	94
47	12,349	3,58	18-Norabietane	107437	1000293-16-6	60
48	13,031	2,11	9,12-Octadecadienoic acid (Z,Z)- methyl ester	132273	000112-63-0	99
49	13,085	4,19	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	130794	000301-00-8	99
50	13,471	1,67	Citronellyl isobutyrate	79642	000097-89-2	52
51	13,740	3,06	Octadecyl trifluoroacetate	180503	079392-43-1	94
52	15,239	1,62	Cyclotetracosane	163070	000297-03-0	99
53	16,137	2,02	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	158684	023470-00-0	74
54	16,514	2,99	1,2,3,4-Tetrahydro-1,4-ethanoanthracene, 9,10-dimethoxy-	111975	177205-54-8	87
55	18,094	7,68	13-Docosenamide, (Z)-	163527	000112-84-5	96
56	18,435	25,59	Squalene	198700	007683-64-9	99
57	21,137	5,05	dl-.alpha.-Tocopherol	203749	010191-41-0	99
58	23,740	3,92	4,4'-(1-(p-(4-Hydroxy-alpha,alpha,-dimethylbenzyl)phenyl)ethylidene)diphenol	202268	110726-28-8	44
59	24,637	6,35	Ergosta-4,6,22-trien-3.beta.-ol	194358	034026-93-2	49
60	25,643	3,07	Methanol, 6,8,9-trimethyl-4-(2-phenylethyl)-3-oxabicyclo[3.3.1]non-6-en-1-yl)-	137035	1000277-01-1	20
61	9,799	2,38	Dodecyl acrylate	90201	002156-97-0	91
62	10,365	0,80	Tetradecanoic acid	81211	000544-63-8	99
63	10,643	1,59	1-Octadecene	99556	000112-88-9	96

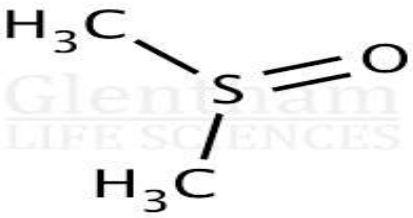
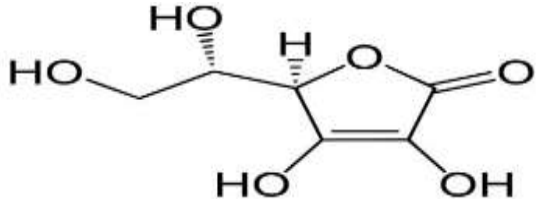
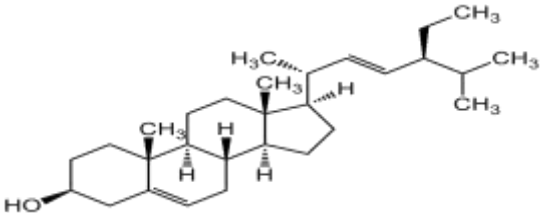
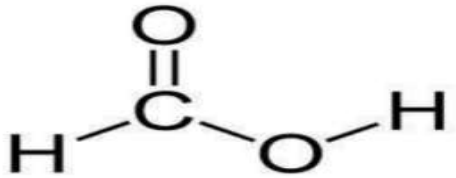
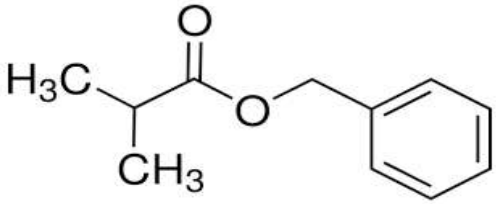
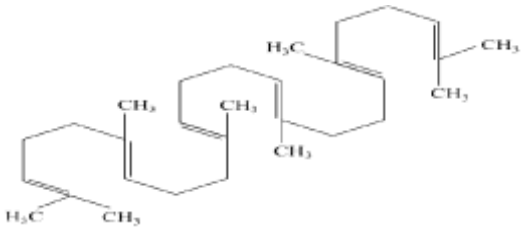
64	11,443	1,03	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0(3,8)]undecane	74833	1000185-86-6	66
65	12,043	19,63	n-Hexadecanoic acid	102726	000057-10-3	99
66	12,223	2,99	Cycloeicosane	121341	000296-56-0	97
67	13,174	1,17	Phytol	133806	000150-86-7	91
68	13,426	19,80	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	119801	000463-40-1	98
69	13,543	0,88	Octadecanoic acid	124559	000057-11-4	78
70	13,749	2,60	Behenic alcohol	156095	000661-19-8	94
71	15,077	0,74	Benzenamine, 4,4'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis-	198641	013080-86-9	43
72	15.239	1,49	1-Docosanol, methyl ether	165737	1000333-91-9	94
73	18,094	4,42	13-Docosenamide, (Z)-	163527	000112-84-5	97
74	21,172	3,27	dl-.alpha.-Tocopherol	203748	010191-41-0	87
75	22,456	0,89	Campesterol	195897	000474-62-4	96
76	22,896	9,50	Stigmasterol	199250	000083-48-7	99
77	23,740	18,76	gamma.-Sitosterol	199879	000083-47-6	99
78	24,539	1,98	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	199891	000521-03-9	87
79	24,871	1,72	4,22-Stigmastadiene-3-one	198681	020817-72-5	99
80	25,912	4,35	Stigmast-4-en-3-one	199256	001058-61-3	95
81	11,783	1,84	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	118306	082304-66-3	99
82	13,166	2,33	Phytol	133804	000150-86-7	87
83	13,740	2,85	Heptadecyl trifluoroacetate	172657	1000351-87-0	94
84	15,239	2,70	Cyclotetracosane	163070	000297-03-0	99
85	16,146	11,18	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	158684	023470-00-0	95
86	16,451	2,77	1,2-Benzenedicarboxylic acid, diisooctyl ester	192052	027554-26-3	68
87	16,702	1,00	Z-5-Nonadecene	110438	1000131-11-8	99
88	17,627	5,59	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	176318	000123-94-4	80
89	18,103	24,41	13-Docosenamide, (Z)-	163527	000112-84-5	99

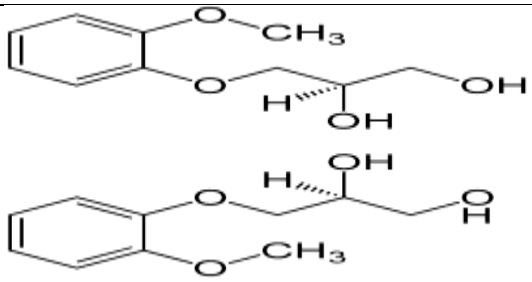
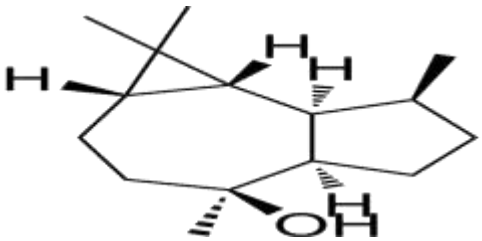
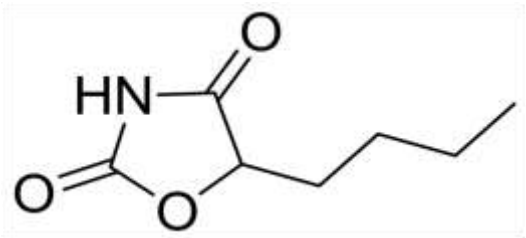
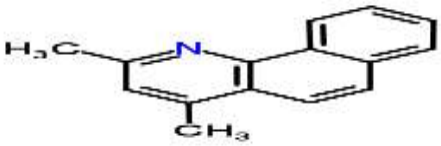
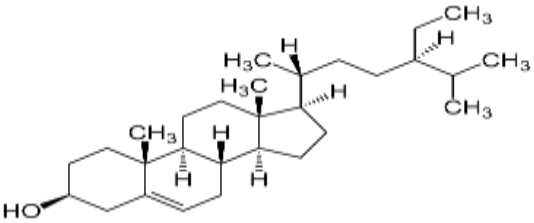
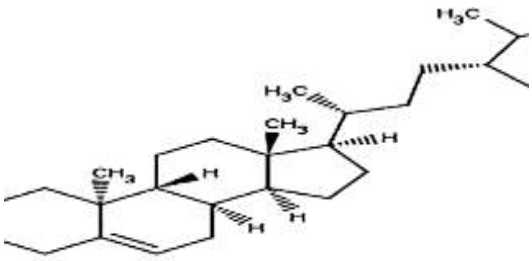
90	18,417	4,46	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	198715	000111-02-4	99
81	18,839	0,99	Eicosane, 9-octyl-	193570	013475-77-9	25
92	21,155	1,96	dl-.alpha.-Tocopherol	203749	010191-41-0	96
93	21,334	1,62	2-(p-(Dimethylamino)phenyl)-1-phenylbenzimidazole	146683	002562-73-4	47
94	22,860	8,45	Stigmasterol	199250	000083-48-7	99
95	23,677	17,31	gamma.-Sitosterol	199879	000083-47-6	99
96	24,521	1,98	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	199891	000521-03-9	94
97	24,853	1,74	4,22-Stigmastadiene-3-one	198681	020817-72-5	38
98	25,059	1,42	1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	64941	1000351-62-2	38
99	25,634	0,93	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	122638	093103-70-9	41
100	25,894	4,48	Stigmast-4-en-3-one	199256	001058-61-3	95
101	7,627	1,48	6-Tridecen-4-yne, (E)-	43073	074744-46-0	30
102	9,072	1,45	1,2,3-Benzenetriol	10981	000087-66-1	38
103	10,643	1,87	1-Nonadecene	110437	018435-45-5	95
104	12,008	20,53	n-Hexadecanoic acid	102726	000057-10-3	99
105	12,223	2,48	Cycloeicosane	121341	000296-56-0	98
106	12,923	1,78	Cyclohexadecane	78201	000295-65-8	64
107	13,174	2,77	Phytol	133806	000150-86-7	91
108	13,339	15,62	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	119801	000463-40-1	99
109	13,516	1,69	Octadecanoic acid	124559	000057-11-4	86
110	13,695	1,87	Hexadecanoic acid, butyl ester	145978	000111-06-8	94
111	13,740	2,09	1-Octadecene	99556	000112-88-9	97
112	16 ;738	2,28	1H-Indole, 5-methyl-2-phenyl-	64835	013228-36-9	49
113	18,093	8,17	13-Docosenamide, (Z)-	163527	000112-84-5	97
114	18,417	6,94	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	198715	000111-02-4	99
115	21,136	3,39	Vitamin E	203745	000059-02-9	99

116	21,334	1,77	Thiophene, 2-(4-methoxyphenyl)-5-(4-methoxyphenylthio)-	157029	107683-45-4	41
117	22,860	5,46	Stigmasterol	199250	000083-48-7	99
118	23,686	12,84	gamma.-Sitosterol	199879	000083-47-6	99
119	24,862	1,67	4,22-Stigmastadiene-3-one	198681	020817-72-5	83
120	25,894	4,04	Stigmast-4-en-3-one	199256	001058-61-3	93
121	7,627	1,48	6-Hexadecen-4-yne, (E)-	74896	074744-52-8	83
122	9,647	0,94	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	76573	000473-15-4	90
123	10,616	1,22	Cyclohexanone, 3-(4-hydroxybutyl) -2-methyl-	47342	091212-98-5	30
124	10,751	1,32	Guaifenesin	7a6182	077846-84-5	72
125	10,975	1,31	Ledol	65640	1000196-01-5	30
126	11,083	0,94	2-Pentadecanone, 6,10,14-trimethyl-	112035	000502-69-2	94
127	13,166	1,93	Phytol	133805	000150-86-7	91
128	13,740	0,97	Octadecyl pentafluoropropionate	200185	1000351-80-7	93
129	15,239	1,54	Cyclotetracosane	163070	000297-03-0	99
130	16,137	6,97	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	158684	023470-00-0	83
131	16,451	1,12	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	119596	004376-20-9	74
132	17,618	2,41	Octadecanoic acid, 2,3-dihydroxypropyl ester	176318	000123-94-4	43
133	18,085	36,42	13-Docosenamide, (Z)-	163527	000112-84-5	97
134	18,417	1,41	4-Geranyloxy-3-hydroxy-5-methoxyphthalaldehyde	160093	076878-70-1	35
135	18,839	1,60	5,5'-Di(ethoxycarbonyl)-3,3' -dimethyl-4,4'-dipropyl-2,2'-dipyrrylmethane	196389	102586-97-0	43
136	19,826	8,08	1,2-Bis(trimethylsilyl)benzene	76049	017151-09-6	38
137	21,155	1,06	Benzo[h]quinoline, 2,4-dimethyl-	64841	000605-67-4	38
138	22,851	8,01	Stigmasterol	199250	000083-48-7	95
139	23,659	17,50	beta.-Sitosterol	199877	000083-46-5	91
140	25,885	3,79	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-	76239	055012-80-1	43

Most of the compounds identified in our ethyl acetate leaf extracts of *Combretum micranthum* G.Don appear to possess medicinal properties and some of them are frequently found in many medicinal plants (Table 3).

Table 3: Common bioactive compounds found in the ethyl acetate leaf extract of *Combretum micranthum* G.Don, identified by GC-MS analysis.

Compounds	Chemical Structure	Percentage of Surface	Mass/Charge Ratio (m/z)	Pharmacological and Medicinal Properties
Phytol		10,46	83,10	Anti-inflammatory Antimicrobial Antioxidant Joint or muscle pain
Octadecyl trifluoroacetate		3,06	83,10	Antimicrobial Prostaglandin E2 inhibitor
Vitamine E		5,44	165,10	Antioxidant Anti-inflammatory Vasoprotective Improves skin and hair health
Stimastérol		9,50	83,10	Anticancer agents Anti-inflammatories Immunomodulators
Acide formique		1,83	69,10	Antiseptics and disinfectants Bactericides Fungicides
Citronelle isobutyrate		1,67	57,10	Perfumes Flavorings Skincare
Squalène		25,59	137,20	Vaccine adjuvants Cosmetics Dietary supplements

Guaifenesin		1,32	79,10	Treatment of chesty coughs associated with respiratory conditions such as colds, flu, bronchitis, and sinusitis. Facilitates mucus expectoration
Ledol		1,31	91,10	Expectorant and cough suppressant Antifungal
13-Docosenamide, (Z)-		36,42	72,10	Antimicrobial Anticancer Anti-inflammatory
Benzo[h]quinoline, 2,4-dimethyl-		1,74	95,10	Antimicrobial Antifungal Antiprotozoal
Beta.-Sitosterol		17,50	107,10	Hypocholesterolemia Prevention of Many cardiovascular diseases Benign prostatic hyperplasia (BPH)
Gamma.-Sitosterol		18,76	329,10	Cholesterol reduction Anti-inflammatory Antioxidant Antidiabetic

DISCUSSION

The use of herbal remedies and their increasing demand have led to extensive research exploring the medicinal potential of various plant species. Numerous phytochemicals and bioactive compounds have been successfully extracted from different parts of the plant, including roots, leaves, and stems, thus contributing significantly to the discovery and development of therapeutic drugs for the treatment and management of a wide range of diseases. This GC/MS characterization revealed the presence of several bioactive compounds, including flavonoids, terpenoids, steroids, glycosides, and alkaloids. These results suggest that ethyl acetate may be a more effective solvent for extracting bioactive compounds from the leaves of *C. micranthum*,

likely due to its polarity and its greater capacity to dissolve a wider range of phytochemicals. Plant phytochemicals constitute a defense mechanism against many microorganisms. The therapeutic properties of medicinal plants are likely due to the presence of various secondary metabolites (Olayaki et al., 2015; Teixeira et al., 2017). Our ethyl acetate extracts from *Combretum micranthum* G.Don leaves revealed the presence of phenols, alkaloids, saponins, terpenoids, and glycosides, compounds known for their therapeutic activity against disease-causing pathogens. Studies by Welch et al. (2018) and Zeitoun et al. (2020) demonstrated the presence of flavonoids in *C. micranthum* leaf extracts. The structure of each compound was elucidated by various spectrometric methods, including UV, MS, and NMR spectroscopy, or compared to commercially available standards by HPLC using a high-resolution quadrupole time-of-flight (QToF) LC-MS instrument, respectively. Alkaloids, the second family of compounds in *C. micranthum* to be studied after flavonoids, were first reported in 1943 by Lehman (unidentified), and were shown years later by Organ (1972). Jibril et al. (2022) also demonstrated the presence of alkaloids in *C. micranthum* leaves. Alkaloids have shown promising antidiarrheal, anti-inflammatory, anticancer, and antidiabetic activities, as well as potential uses in the treatment of urinary disorders (Awoyinka AO et al., 2007; Ebrahimzadeh et al., 2008; Paliwal et al., 2011). Steroids are natural lipids, or fat-soluble chemical compounds, with a wide range of physiological activities. Therefore, a qualitative test could prove relevant for the discovery of bioactive components.More than a hundred biomolecular compounds were detected in our various ethyl acetate leaf extracts of *Combretum micranthum* G.Don. The compounds 1-octadecene (5.25%), vitamin E (5.44%), cholest-7-en-3-one, 4,4-dimethyl- (5.56%), octadecanoic acid, 2-hydroxy-1 (hydroxymethyl)ethyl ester (5.59%), 1-docosene (5.64%), hexadecanoic acid, methyl ester (5.69%), ergosta-4,6,22-trien-3β-ol (6.35%), cycloeicosane (6.65%), 9,19-cyclolanost-24-en-3-ol, (3β)- (7.21%), 1-nonadecene (7.85%), 1,2-bis(trimethylsilyl)benzene (8.08%), stigmasterol (9.50%), phytol (10.46%), stigmast-4-en-3-one (12.34%), 9,12,15-octadecatrienoic acid (Z,Z,Z) (15.62%), β-sitosterol (17.50%), 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E) (17.98%), γ-sitosterol (18.75%), n-hexadecanoic acid (20.53%), 9,12,15-octadecatrienoic acid (Z,Z,Z) (19.80%), squalene (25.59%), 13-docosenamide (Z) (36.42%) were predominant (≥ 5%). The following compounds, including behenic alcohol, 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-, phytol, 9,12,15-octadecatrienoic acid, (Z,Z,Z)-, vitamin E, stigmasterol, gamma-sitosterol, stigmast-4-en-3-one, 1-octadecene, beta-sitosterol, 1-nonadecene, 18-norabietane, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 13-docosenamide, (Z)-, dl-alpha-tocopherol, stigmast-7-en-3-ol, (3.beta.,5.alpha.)-, cyclotetracosane, stigmast-7-en-3-ol, (3.beta.,5.alpha.)-, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, cholest-7-en-3-one, 4,4-dimethyl-, 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-, 18-norabietane, and 4,22-stigmastadiene-3-one were identical, as expected, by comparison with the National Institute of Standards and Technology (NIST) databases. However, they differed in their respective retention times, surface area, and percentage of surface area. This can be explained by the fact that we worked with different subfractions of the same ethyl acetate extract. Thirteen common bioactive compounds present in our *C. micranthum* leaf extracts, their chemical structure, surface area percentage, mass-to-charge ratio, and pharmacological and medicinal effects are listed in Table 3. These results show that vitamin E (5.44%), stigmasterol (9.50%), phytol (10.46%), β-sitosterol (17.50%), γ-sitosterol (18.75%), squalene (25.59%), and 13-docosenamide (Z)- (36.42%) were the predominant compounds in our various extracts. This confers upon these compounds various therapeutic and pharmaceutical benefits, such as the hydroxylation of liver enzymes, antipyretic and analgesic properties, antirheumatic and antimicrobial activity during phase I metabolism, stimulation of hair growth, inhibition of uric acid production, and inhibition of arachidonic acid. in the organism (Florence and Jeeva, 2015). Our results are consistent with those found by Welch et al. (2010), who identified approximately one hundred biomolecular compounds and dozens of bioactive compounds. The presence of some of these bioactive compounds confirms similar findings by Tine Y et al. (2024), who reported that *Combretum micranthum* G.Don contains tannins, phenols, glycosides, alkaloids and terpenoids, steroids, amino acids, and other organic compounds among its phytochemicals. Previous studies, based on GC-MS analysis of ethanolic extracts of *Combretum micranthum* G.Don leaves, identified 9-octadecene-1-ol, cis-9-octadecene-1-ol, oleol, satol, ocenol, sipo, decanoic acid, and dodecanal as the major compounds (Okwu and Ighodaro, 2010), which is consistent with the results of the present study.

CONCLUSION

Available scientific research on *C. micranthum* has shown that it is a medicinal plant widely used in traditional medicine for treating conditions such as diabetes, fever, cough, bronchitis, diarrhea, pain, malaria, and liver disorders. This plant has a long history of use without any adverse effects that have been proven and documented to date. Phytochemical analyses have revealed the presence of various families of compounds, including tannins, phenolic acids, alkaloids, fatty acids, terpenoids, steroids, amino acids, carbohydrates, vitamins, and minerals. Different extracts of *C. micranthum* leaves possess a wide range of pharmacological properties, including antibacterial, antiviral, antioxidant, antidiabetic, anti-inflammatory, analgesic, antihypertensive,

nephroprotective, hepatoprotective, anxiolytic, anticholinesterase, and antidiarrheal effects. These pharmacological properties could be attributed to the presence of various phytochemicals; however, most pharmacological studies have been conducted using uncharacterized crude extracts of *C. micranthum*. Factors such as geographical and seasonal variations play a significant role in identifying the chemical constituents responsible for pharmacological activity. The results of GC/MS characterization indicate the rich bioactive profile of *C. micranthum* extracts, and ethyl acetate proves to be a particularly effective solvent for the extraction of these compounds. The bioactive constituents identified in this study have been extensively documented for their important pharmacological and medicinal properties. These results reinforce the potential of *C. micranthum* in the management of diseases and underscore the need for a thorough pharmacological exploration of its bioactive compounds to advance drug discovery.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this work.

CONTRIBUTION OF AUTHORS

MElBN and DBC initiated the research on the characterization of biomolecular compounds in powdered leaf extracts of Combretum micranthum G.Don. MElBN wrote the article. SOS supervised the laboratory work. YMD and BYK authorized the laboratory work. SOS, DF, YMD, BYK, and AD facilitated the implementation of the work. AD reviewed and corrected the article.

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