

Research Article

Phytochemical Profiling and Health Benefits of Chloroform and Methanol Extracts of *Laurus nobilis* (Bay Leaf)

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Abstract

Background: *Laurus nobilis*, commonly known as bay leaf, is widely used in global cuisine for flavouring soups and stews, as well as in baked foods and desserts. The present study aims to characterize the phytochemical composition of chloroform and methanol extracts of *Laurus nobilis* using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. **Materials and Methods:** Dried Bay leaves were locally sourced, properly identified, and authenticated. The leaves were extracted using cold maceration to obtain chloroform (CELN) and methanol (MELN) extracts of *Laurus nobilis*. Qualitative and quantitative phytochemical screening, along with Gas Chromatography-Mass Spectrometry (GC-MS) analysis, was performed following standard protocols. **Results:** The qualitative analysis of CELN and MELN confirmed the presence of flavonoids, phenols, terpenoids, glycosides, steroids, saponins, alkaloids, and carbohydrates. Quantitative analysis indicated that MELN contained higher levels of phenols (11.34 mg/100g), tannins (5.20 mg/100g), and carbohydrates (16.23 mg/100g). GC-MS analysis identified 87 and 98 compounds in CELN and MELN, respectively, with 10 compounds common to both extracts. The most abundant ($\geq 5\%$) compounds in MELN were Spiro(1,3,3-trimethylindoline)-2,5'-pyrrolidin-2-one (8.35%), 7,10,13-Hexadecatrienoic acid, methyl ester (12.75%), Azuleno(4,5-b)furan-2(3H)-one, 3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9-bis(methylene) (9.09%), and n-Hexadecenoic acid (18.25%). For CELN, the most abundant compounds were Buta-1,3-diyne,1,4-bis(2-methoxycarbonylcyclopropyl) (5.11%), Azuleno[6,5-b]furan-2,5-dione, decahydro-4a,8-dimethyl-3-methylene-,3aR-(3a α ,4a,7a α ,8 β ,9a α) (5.75%), n-Hexadecanoic acid (5.89%), phytol (7.57%), and Benzene, 1-phenyl-4-(2,2-dicyanoethenyl) (13.91%). **Conclusion:** This study highlights the rich phytochemical and bioactive profile of *Laurus nobilis* (bay leaf) extracts, reinforcing their potential in disease management. It also underscores the need for comprehensive pharmacological investigations of its bioactive compounds to support drug discovery efforts.

Keywords

Laurus nobilis, Bay Leaf, Phytochemical, Bioactive Compounds, Gas Chromatography-mass Spectrometry

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1. Introduction

Traditional medicine, as it is practiced today, has its roots in the utilization of natural resources, primarily derived from plants as various communities across the world have historically relied on these natural remedies out of necessity to manage and prevent a wide range of diseases [1-3]. This practice has been shaped by generations of empirical knowledge, observation, and cultural transmission [4]. Ethnobotanical studies have shown that indigenous and local communities have developed extensive pharmacopoeias based on the medicinal properties of plants, which have served as the foundation for many modern pharmaceuticals [5, 6]. Plant-derived natural compounds have garnered significant interest in recent years due to their diverse pharmacological properties [7, 8]. The widespread use of herbal products in primary healthcare is largely attributed to their accessibility, affordability, and relatively low incidence of adverse effects compared to synthetic drugs [1, 9]. Approximately half of the world's population depends on traditional medicine and herbal remedies as their primary healthcare option, especially in developing nations where access to conventional medical treatments may be restricted or financially burdensome [4, 10].

Laurus nobilis, commonly known as Bay leaf is a perennial shrub that belongs to the laurel family (Lauraceae). They are widely used in global cuisine for their aromatic and flavor-enhancing properties where they are typically added to dishes whole and removed before serving, as their tough texture makes them unsuitable for direct consumption [11, 12]. They are specifically used in flavouring soup and stews, marinating and enhancing meat and poultry dishes, seasoning rice and other grain dishes, infused in oils and vinegars as well as incorporated into bakeries and deserts [12-15]. Although widely employed as a culinary herb, they also possess significant medicinal and pharmacological properties. Its bioactive compounds, including essential oils, triterpenoids, steroids, flavonoids, alkaloids, and tannins [15, 16] give them a wide range of pharmacological benefits. *Laurus nobilis* leaf extracts have been shown to possess anti-inflammatory [17, 18], analgesic [19], antioxidant [20, 21], antimicrobial [22, 23], antifungal [23], gastroprotective [24, 25], hypoglycemic [26, 27], hypolipidemic [28, 29], immunomodulatory [30, 31], neuroprotective [32, 33] and anticancer [34, 35] potentials.

In recent years, there has been a growing interest in phytochemical research, with an emphasis on isolating, identifying, and characterizing bioactive compounds from plants. This field serves as a crucial foundation for drug discovery, development, and further scientific investigations [1, 36]. While several studies investigated the phytochemical profile and therapeutic potential of *Laurus nobilis*, there remains a limited understanding of the specific chemical constituents present in different solvent fractions. Chloroform and methanol possess different polarities, which may selectively extract distinct classes of bioactive compounds that have not

been extensively characterized. However, there is limited comparative research on how these solvents impact the chemical profile and pharmacological potential of bay leaf extracts. The present study is therefore focused on the qualitative and quantitative characterization of chloroform and methanol extracts of *Laurus nobilis* by the method of phytochemical extraction and Gas chromatography-mass spectrometry (GC-MS) analysis. This will provide deeper insights into the diverse chemical profile of *Laurus nobilis*, facilitating its potential application in drug discovery and alternative medicine.

2. Materials and Methods

2.1. Plant Source and Identification

Dried bay leaves were obtained from a local herb store at Mile 3 Market, Port Harcourt, Nigeria. The leaves were identified and authenticated by Edwin Nwosu from the Department of Plant Science and Biotechnology, University of Port Harcourt, and a voucher specimen (ONN/001) was deposited at the Ecoright Herbarium.

2.2. Plant Preparation and Extraction

The dried leaves of *Laurus nobilis* were pulverized using an electric blender, and the resulting coarse powder was divided into two equal portions, each weighing 650 g. They were extracted using the cold maceration technique, following previously established procedures [37, 38]. Each portion was separately macerated in 2.4 L of methanol and chloroform in glass jars for three days, with intermittent stirring. The mixtures were then filtered using a filter paper, and the filtrates were concentrated at 45 °C under reduced pressure using a rotary vacuum evaporator. The concentrated extracts were transferred into crucibles and further dried at 90 °C using a water bath until dry solid pastes were obtained, yielding 20.4 g of methanol extract and 19.26 g of chloroform extract. The extracts were stored at 4 °C until further phytochemical screening.

2.3. Phytochemical Screening Test

Chloroform extracts of *Laurus nobilis* (CELN) and methanol extracts of *Laurus nobilis* (MELN) were subjected to qualitative phytochemical analysis to identify various phytoconstituents following the standard protocols by Trease and Evans [39]. For these tests, 0.5g CELN and MELN were dissolved in 10 ml of distilled water to obtain a diluted extract solution of CELN and MELN respectively and 2 ml of the extract solutions were used for the test.

Flavonoids Test: The Shinoda Test method was used to test for flavonoids by adding 2 ml of the extract solution to a small

amount of magnesium ribbon and a few drops of concentrated hydrochloric acid added. The appearance of a pink, red, or orange colouration confirms the presence of flavonoids.

Phenols Test: The Ferric Chloride Test method was used to test for phenols by adding 2 ml of the extract solution to 2-3 drops of 5% ferric chloride solution. The formation of a blue, green, or black colour indicates the presence of phenols.

Terpenoids Test: The Salkowski Test method was used to test for terpenoids by adding 2ml of extract solution to 2ml of concentrated sulfuric acid carefully along the side of the test tube. The appearance of a reddish-brown interface confirms the presence of terpenoids.

Glycosides Test: The Keller-Killiani Test method was used to test for glycosides by adding 2 ml of extract solution to glacial acetic acid containing one drop of ferric chloride solution. About 1 ml of concentrated sulfuric acid was then carefully added. The appearance of a brown ring at the interface confirms the presence of glycosides.

Steroids Test: The Liebermann-Burchard Test method was used to test for steroids by adding 2 ml of extract solution to 1 ml of acetic anhydride and 2 ml of concentrated sulfuric acid. The appearance of a green or bluish-green colouration confirms the presence of steroids.

Saponins Test: The Foam Test was used to test for saponins by shaking 5 ml of the extract solution vigorously for 5 minutes. The appearance of persistent frothing indicates the presence of saponins.

Alkaloids Test: Mayer's Test was used to test for alkaloids by adding 2 ml of the extract mixture to Mayer's reagent (potassium mercuric iodide). The appearance of a creamy white precipitate confirms the presence of alkaloids.

Tannins Test: The Ferric Chloride Test was used to test for tannins by adding 2-3 drops of the 5% ferric chloride solution to 2 ml of extract solution. The appearance of a blue-black or green precipitate indicates the presence of tannins.

Carbohydrates Test: The Molisch's Test was used to test for carbohydrates by adding 2 ml of extract solution to 2 drops of Molisch's reagent and thoroughly mixed followed by the careful addition of 1 ml of concentrated sulfuric acid along the tube wall. The appearance of a reddish-violet ring at the interface confirms the presence of carbohydrates.

2.4. Quantitative Phytochemical Analysis

The MELN and CELN were subjected to quantitative chemical analysis to determine the concentrations of various phytochemicals using standardized methods as outlined by Harborne, J. B [40] and previously described [1]. The principle is based on the chemical properties of the compounds and their reactions with specific reagents to produce colours and or precipitates with absorbance measured against a standard to determine the concentration.

Flavonoids: The extracts were mixed with an aluminium chloride solution and ethanol, resulting in the formation of a yellow-coloured complex with flavonoids. The absorbance of

this complex was measured at 420 nm using a spectrophotometer. The concentration of flavonoids was determined using a standard curve prepared with quercetin.

Phenols: The extracts were mixed with Folin-Ciocalteu reagent, producing a blue-coloured complex. The absorbance was measured at 760 nm, and the concentration was determined using a standard curve based on gallic acid.

Terpenoids: The extracts were mixed with acetic anhydride and sulfuric acid in the Liebermann-Burchard test to form green or blue-green coloured complexes. The absorbance was measured at 538 nm and the concentration was determined using a standard curve using linalool as reference.

Glycosides: The extracts were hydrolyzed with concentrated sulfuric acid to release aglycones, which were then reacted with acetic anhydride to form coloured complexes, typically reddish-brown or blue. The absorbance was measured at 620 nm, and the concentration was calculated using a standard curve based on digoxin.

Steroids: The extracts were mixed with acetic anhydride and sulfuric acid to form a green-coloured complex. The absorbance was measured at 620 nm and the concentration was determined using a standard curve based on cholesterol.

Saponins: The extracts were reacted with vanillin and sulfuric acid, resulting in the formation of a red-coloured complex. The absorbance was measured at 560 nm, and the concentration was calculated using a standard curve based on digitonin.

Alkaloids: the extracts were treated with bromocresol green, which forms a yellow-coloured complex with alkaloids. The absorbance of the complex was measured at 470 nm, and the concentration was calculated using a standard curve based on quinine as the reference standard.

Tannins: The extracts were combined with Folin-Denis reagent, which reacts with tannins to produce a blue colour. The absorbance was measured at 760 nm, and the concentration was determined using a standard curve based on tannic acid.

Carbohydrates: The extracts were mixed with anthrone reagent to form coloured complexes. The absorbance was measured at 620 nm and the concentration was quantified using a standard curve based on glucose as a reference.

2.5. Chemical Characterization by Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was conducted using an Agilent 5973N instrument equipped with a capillary column (Agilent HP-5MS UI). The temperature program began at 60 °C, held for 5 minutes, and was then gradually increased to 180 °C and further to 280 °C, with each temperature held for 10 minutes. Helium served as the carrier gas, and the injection was performed in split mode at a 1:30 ratio. Mass spectra were acquired in the range of m/z 30–500 using an ionizing voltage of 70 eV. The Kovats retention index was calculated using

standard hydrocarbons for reference. The compounds were identified by analyzing their retention times and fragmentation patterns using the mass spectra generated from the GC-MS analysis. The active components in the extract were further verified by comparing their retention indices, peak area percentages, and mass spectral fragmentation patterns 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 [1].

3. Results

Table 1. Qualitative phytochemical screening of chloroform and methanol extracts of *Laurus nobilis* leaves.

Phytochemical	CELN	MELN
Flavonoid	+	+
Phenol	+	+
Terpenoids	+	+
Glycoside	+	+
Steroid	+	+
Saponin	+	+
Alkaloid	+	+
Tannin	+	+
Carbohydrate	+	+
present +		

Table 1 shows the presence of various phytochemicals found in chloroform and methanol extracts of *Laurus nobilis* leaf. The results reveal that the extracts contain significant quantities of flavonoids, phenols, terpenoids, glycoside, steroids, saponins, alkaloids and carbohydrates.

Table 2. Quantitative phytochemical screening of chloroform and methanol extracts of *Laurus nobilis* leaves.

Phytochemicals	CELN (mg/100g)	MELN (mg/100g)
Flavonoid	3.61	3.58
Phenol	3.23	11.34
Terpenoids	6.56	6.24
Glycoside	1.24	1.18
Steroid	0.51	0.61
Saponin	0.26	0.24
Alkaloid	0.14	0.16
Tannin	1.35	5.20
Carbohydrate	7.10	16.23

Table 2 presents the concentrations of various phytochemicals, measured in milligrams per 100 grams (mg/100g), identified in the chloroform and methanol leaf extracts of *Laurus nobilis*. The data indicate that the methanol extract exhibited significantly higher levels of key phytochemicals compared to the chloroform extract. Specifically, the methanol extract contained elevated concentrations of phenols (11.34 mg/100g), tannins (5.20 mg/100g), and carbohydrates (16.23 mg/100g).

Table 3. Characterization of chloroform extract of *Laurus nobilis* leaf by GC-MS analysis.

Peak Number	Retention Time	Area Time	Area %	Compound
1	0.811	3008182	0.07	Ethane,1-bromo-2-chloro
2	1.157	4857787	0.12	1,3-Propanediol,2-amino-1-(4-nitrophenyl)
3	1.214	2546564	0.06	D-Mannoheptulose
4	1.253	1435874	0.03	2(R),3(S)-1,2,3,4-Butanetetrol
5	1.308	3698708	0.09	11-Bromoundecanoic acid
6	1.369	581024	0.01	p-Dioxane-2,3-diol
7	9.345	5151875	0.12	4-Fluorobenzyl alcohol
8	9.400	5227954	0.13	Cyclohexane, isocyanato-
9	9.429	6781833	0.16	1-Azabicyclo [2.2.2] octan-3-one
10	9.701	5116255	0.12	Methyl 2-furoate

Peak Number	Retention Time	Area Time	Area %	Compound
11	9.974	8264848	0.20	Methyl m-tolyl carbinol
12	10.184	22762988	0.55	Eugenol
13	10.648	17183421	0.41	Benzene, 1,2-dimethoxy-4-(2-propenyl)
14	10.829	4575630	0.11	Naphthalene, 2,7-dimethyl
15	11.004	13045282	0.31	Naphthalene, 2,7-dimethyl-
16	11.200	8296130	0.20	3-Allyl-6-methoxyphenol
17	11.573	7023918	0.17	2-Naphthalenamine, 1,2,4a,5,6,7,8,8a-octahydro-4a-methyl
18	11.606	4615619	0.11	Benzene, 1,2-dimethoxy-4-(1-propenyl)
19	11.642	5416863	0.13	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7- (1-methylethenyl)
20	11.985	20086280	0.48	2H-1-Benzopyran-2-one,7,8-dihydroxy-6-methoxy
21	12.034	12000733	0.29	2,4,4-Trimethyl-3-(3-methylbutyl) cyclohex-2-enone
22	12.070	12313996	0.30	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran
23	12.135	15463330	0.37	m-Xylene- α , α -dithiol
24	12.189	18205803	0.44	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydro-1-benzofuran
25	12.286	11953887	0.29	1H-Indole-2,3-dione, 7-fluoro-
26	12.337	35495123	0.86	Propan-1-one, 1-[4-(1-methylethyl)-2-nitrosophenyl]
27	12.476	8459475	0.20	1-Cyclohexene-1-methanol
28	12.548	4637964	0.11	3-buten-2-one,4-(5,5-dimethyl-1-oxaspiro [2,5] oct-4-yl
29	12.611	13608025	0.33	Acetic acid, 2,6,6-trimethyl-3-methylene-7-(3-oxobutylidene) oxepane-2-yl ester
30	12.709	13190332	0.32	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)
31	12.815	49778025	1.20	Benzene methanol, 2,4-dimethyl-
32	12.995	86277176	2.08	2-Naphthalenemethanol, decahydro- α , α , 4a-trimethyl-8-methylene
33	13.127	23710126	0.57	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)
34	13.276	14062328	0.34	Vitamin A aldehyde
35	13.301	10021927	0.24	Bicyclo [6.1.0] non-1-ene
36	13.495	10337093	0.25	Spiro [4.5] decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl
37	13.600	26923226	0.65	Tricyclo [5.2.2.0(1,6)] undecane-3-ol,2-methylene-6,8,8-trimethyl
38	13.643	31445641	0.76	2,9-Heptadecadiene-4,6-diyn-8-ol, (Z, E)
39	13.927	21968592	0.53	4-Hexen-1-ol,6-(2,6,6-trimethyl-1-cyclohexenyl)-4-methyl
40	14.087	48133156	1.16	3,4-Dimethoxy-6-amino toluene
41	14.256	52266600	1.26	2-(4a,8-Dmethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2yl)-prop-2-en-1-ol
42	14.304	8605359	0.21	3-Chlorobicyclo (2.2.1) heptan-2-one oxime
43	14.351	29004120	0.70	1,4-Methanoazulen-7-ol, decahydro-4,8,8,9-tetramethyl
44	14.390	16038041	0.39	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1Z,3 α , 7 α)
45	14.479	48231296	1.16	Benzene, 1-(bromomethyl)-4-(1-methylethyl)-
46	14.649	65755712	1.59	Pentadecanoic acid, 14-methyl-, methyl ester
47	14.701	13677417	0.33	Caryophyllene-(II)
48.	14.724	7956616	0.19	Bicyclo [5.1.0] octan-2-one,4,6-disopropylidene-8,8-dimethyl

Peak Number	Retention Time	Area Time	Area %	Compound
49	14.759	33314061	0.80	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo [5.4.0.0(3,8)] undecane
50	14.892	30613192	0.74	3,6-Nonadien-5-one,2,2,8,8-tetramethyl
51	15.252	756133195	18.25	n-Hexadecanoic acid
52	15.339	13422097	0.32	6H-Indolo[3,2,1-de][1,5]naphthyr...
53	15.418	48017569	1.16	3H-Naphtho[2,3-b]furan-2-one, 4-...
54	15.519	40862843	0.99	1H-2,6-Methano-2,3-benzodiazocin-8-ol,3,4,5,6-tetrahydro-3,6,11-trimethyl
55	15.626	89157674	2.15	3(4H)-Phenanthrenone, 4a, 4b,5,6,7,8,8a,9,10,10a-decahydro-4b,8,8-trimethyl
56	15.700	78104402	1.89	Isoaromadendrene epoxide
57	15.919	376486592	9.09	Azuleno[4,5-b] furan-2(3H)-one, 3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9bis(methylene)
58	15.986	95209816	2.30	Azuleno[4,5-b] furan-2(3H)-one, 3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9bis(methylene)
59	16.068	16786026	0.41	Ethyl 5,8,11,14,17-eicosapentaenoate
60	16.347	527432505	12.73	7,10,13-Hexadecatrienoic acid, methyl ester
61	16.391	81432636	1.97	9,12,15-Octadecatrienoic acid, (Z, Z, Z)
62	16.439	89760565	2.17	Octadecanoic acid
63	16.465	24653128	0.60	Piperidine, 1-(1-oxo-3-phenyl-2-propenyl)-
64	16.761	10630244	0.26	Propanoic acid, 2-methyl-, (decahydro-6a-hydroxy-9a-methyl-3-methylene-2,9dioxoazuleno[4,5-b] furan-6-yl) methyl ester
65	16.785	5441689	0.13	4-Methyl-3-(3-nitrophenyl)-6-phenyl-5,6-dihydro4H- [1,2,4,5] oxatriazine
66	16.809	6331608	0.15	Vitamin A aldehyde
67	16.899	33345261	0.80	Retinoic acid
68	17.046	47774015	1.15	Azulene, 1,2,3,4,5,6,7,8-octahyd...
69	17.227	148742319	3.59	7-Methyl-5-oxo-2-phenyl-3,5-dihydro-indolizine-6-carbonitrile
70	17.278	44285027	1.07	1H-Cycloprop[e]azulene, decahydro-1,1,4,7-tetramethyl
71	17.475	345965480	8.35	Spiro[1,3,3-trimethylindoline]-2,5'-pyrrolidin-2-one
72	17.621	48129354	1.16	1-Acetylpyrene
73	17.751	112838522	2.72	Buta-1,3-diyne, 1,4-bis(2-methoxycarbonylcyclopropyl)
74	17.895	27759994	0.67	Coumarin-6-ol, 4,4,7-trimethyl-5-nitro-3,4-dihydro-
75	17.977	11063078	0.27	2-Furancarboxylic acid, 5-(4-amino-2-chlorophenyl)-, methyl ester
76	18.108	4750667	0.11	1-Docosanol, formate
77	18.356	4974185	0.12	4-(4-Methoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline
78	18.413	5044550	0.12	Phthalic acid, neopentyl octyl ester
79	19.072	3230939	0.08	N-(4-Isopropylbenzyl)-3-phenylpropionamide
80	19.300	5482330	0.13	Tetracosanoic acid, methyl ester
81	19.539	4723021	0.11	[2-(4-methoxyphenyl)-[benzo(f)(1-nickela-2,3-diazaindene)] -cyclopentadienyl
82	19.596	3298926	0.08	Silane, (estra-1,3,5(10),16-tetraen-3-yloxy) trimethyl-
83	19.964	38356051	0.93	3-Acetyl-1-(4-iodophenyl)-5-phenyl-4,5-dihydro-1H- [1,2,4] triazine-6-one

Peak Number	Retention Time	Area Time	Area %	Compound
84	20.085	47762010	1.15	Bis(2'-hydroxy-3'-isopropylisobutyrophenonato) beryllium(ii)
85	20.184	3025442	0.07	Bis(2'-hydroxy-3'-isopropylisobutyrophenonato) beryllium(ii)
86	21.993	12451079	0.30	Vitamin E
87	24.462	6996235	0.17	β -Sitosterol

Table 3 lists the biomolecular compounds identified in the chloroform extract of *Laurus nobilis* leaf by GC-MS analysis. Eighty-seven (87) compounds were detected. The five most abundant ($\geq 5\%$) compounds are Spiro (1,3,3-trimethylindoline]-2,5'-pyrrolidin-2-one (8.35%),

7,10,13-Hexadecatrienoic acid, methyl ester (12.75%), Azuleno (4,5-b) furan-2(3H)-one, 3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9bis(methylene) (9.09%) and n-Hexadecenoic acid (18.25%).

Table 4. Characterization of methanol extract of *Laurus nobilis* leaf by GC-MS analysis.

Peak number	Retention time	Area time	Area %	Compound
1	0.796	3046906	0.09	1-Chloroethyl sulfone
2	0.848	1791814	0.05	Methylene Chloride
3	0.925	2036079	0.06	Trichloroacetic acid, 2-chloroethyl ester
4	8.189	21496905	0.63	Bicyclo [4.1.0] hept-2-ene, 3,7,7-trimethyl
5	9.603	13707160	0.40	Bicyclo [4.4.1] undeca-1,3,5,7,9-pentaene
6	10.015	130518984	3.83	3-Cyclohexane-1-methanol, α , α , 4-trimethyl-, acetate
7	10.196	42323632	1.24	Eugenol
8	10.658	46358985	1.36	Benzene, 1,2-dimethoxy-4-(2-propenyl)
9	10.948	17898517	0.53	Pyrazine, 2-methoxy-3-(1-methylethyl)-
10	11.089	4377074	0.13	2-Propen-1-ol, 3-phenyl-, acetate
11	11.209	7324346	0.22	Propenoic acid, 3-(2-thienyl)-4-nitrophenyl ester
12	11.423	7136998	0.21	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene
13	11.560	9607464	0.28	Ethanone, 1-(1,4-dimethyl-3-cyclohexane-1-yl)
14	11.602	3792058	0.11	Benzene, 1,2-dimethoxy-4-(1-propenyl)
15	11.640	4358674	0.13	Cyclohexane, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene
16	11.784	5317712	0.16	1,2-Ethanediol, 1,2-dimyrtenyl-
17	11.840	5934614	0.17	Ethanone, 1-(1,4-dimethyl-3-cyclohexan-1-yl)-
18	11.993	28891803	0.85	Benzene, 1,2,4-trimethoxy-5-(1-propenyl)-, (Z)-
19	12.044	5985000	0.18	2H-1,2,3,4-Tetrazole-2-acetamide, N-(1-ethyl-1-methyl-2-propynyl)-5-(2-thienyl)-.
20	12.279	5055948	0.15	Ethanone, 1-(3,5-dimethylpyrazinyl)-
21	12.336	22039135	0.65	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1 $\alpha\alpha$,4 $\alpha\alpha$,7 β ,7 $\alpha\beta$,7 $\beta\alpha$)]-
22	12.363	8929863	0.26	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo [5.4.0.0(3,8)]undecane.
23	12.471	5092726	0.15	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo [7.2.0]undec-3-ene

Peak number	Retention time	Area time	Area %	Compound
24	12.569	4286747	0.13	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo [5.3.1.0(4,11)] undec-8-ene
25	12.604	6337061	0.19	1,3,5,6-Tetramethyladamantane
26	12.699	6304228	0.19	Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethyenyl)
27	12.815	21866551	0.64	10,10-Dimethyl-2,6-dimethylenebicyclo [7.2.0] undecane-5 β -ol
28	12.850	9318615	0.27	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-
29	12.980	46385244	1.36	2-Naphthalenemethanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene-, [2R-(2 $\alpha,4\alpha,8\alpha\beta$)]-
30	13.043	6004299	0.18	1,4-Dimethyl-8-isopropylidenetricyclo[5.3.0.0(4,10)]decane
31	13.081	3293043	0.10	α -Farnesene
32	13.121	5730777	0.17	Isoaromadendrene epoxide
33	13.247	13733636	0.40	Benzene, 1-(2-chloroethyl)-2-(trifluoromethyl)
34	13.291	3717824	0.11	Spiro [2.5] octane, 5,5-dimethyl-4-(3-oxobutyl)
35	13.359	3555832	0.10	Bicyclo [7.2.0] undec-4-ene,4,11,11, trimethyl-8-methylene
36	13.387	2606515	0.08	Isoaromadendrene epoxide
37	13.425	3122097	0.09	1,1-Dichloro-2-methyl-3-(4,4-diformyl-1,3-butadien-1-yl) cyclopropane
38	13.486	2570119	0.08	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1 $\alpha,4\alpha,7\alpha\beta,7b\alpha$)]
39	13.571	19720689	0.58	2H-2a,7-Methanoazuleno[5,6-b] oxirene, octahydro-3,6,6,7a-tetramethyl
40	13.634	2261804	0.07	Cycloheptane,4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl
41	13.717	5159902	0.15	Benzoic acid, 2-amino-3-hydroxy
42	13.917	8726130	0.26	Felbinac
43	14.052	16404823	0.48	s-Triazolo[4,3-a] pyrazine,5,8-dimethyl-3-(methylthio)
44	14.169	2472656	0.07	Cyclopropa [c, d]pentalene-1,3-dione, hexahydro-4-(2-methyl-2-propenyl)-2,2,4-trimethyl
45	14.215	17355823	0.51	Longifolenaldehyde
46	14.312	21526835	0.63	2-Hydroxy-2,4,4-trimethyl-3-(3-methylbuta-1,3-dienyl) cyclohexnone
47	14.460	24206192	0.71	1,3,4-Oxadiazole,2-[3-(4-fluorophenyl)-1H-pyrazol-5-yl]-
48	14.647	45080147	1.32	trans-Z- α -Bisabolene epoxide
49	14.713	12829679	0.38	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene
50	14.754	17032444	0.50	Calarene epoxide
51	14.797	15221631	0.45	5-Isopropylidene-6-methyldeca-3,6,9-trien-2-one
52	14.878	21600102	0.63	1H-Indene,1-ethylideneoctahydro-7a-methyl-, (1E,3 $\alpha,7\alpha\beta$)
53	15.005	138517678	4.07	p-(m-Hydroxyphenoxy)benzoic acid
54	15.052	33936869	1.00	4H-3,1-Benzoxazine,1,2,4arel,5trans, 6,7,8trans,8acis-octahydro-5,8-methano-1methyl-2(phenylimino)
55	15.080	27543426	0.81	n-Hexadecanoic acid
56	15.190	200632389	5.89	n-Hexadecanoic acid
57	15.237	26771143	0.79	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethyenyl)-, [1R-(1 $\alpha,7\beta,8\alpha\alpha$)]-
58	15.330	18885633	0.55	Caryophyllene oxide

Peak number	Retention time	Area time	Area %	Compound
59	15.405	36689855	1.08	17-Octadecene-9,11-diynoic acid, 8-oxo-, methyl ester
60	15.496	24234808	0.71	11H-Indeno(1,2-b)quinoline
61	15.633	125684528	3.69	Ambrosin
62	15.693	32670814	0.96	1,4-Methanoazulene decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1 α ,3 $\alpha\beta$,4 α ,8 $\alpha\beta$)]
63	15.919	257517836	7.57	Phytol
64	15.941	68359838	2.01	Azuleno [4,5-b] furan-2(3H)-one,3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9-bis(methylene)-[3As-(3 $\alpha\alpha$,6 $\alpha\alpha$,9 $\alpha\alpha$,9b β)]-
65	15.999	130236207	3.83	Azuleno [4,5-b] furan-2(3H)-one,3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9-bis(methylene)-[3As-(3 $\alpha\alpha$,6 $\alpha\alpha$,9 $\alpha\alpha$,9b β)]-
66	16.049	7118677	0.21	N-(3,4,5-Trimethoxybenzylidene) isopropylamine
67	16.101	11001444	0.32	6-Methyl-3,3'-bi(1H-indole)
68	16.279	160337905	4.71	9,12-Octadecadienoic acid (Z,Z)-
69	16.306	45855473	1.35	7,10,13-Hexadecatrienoic acid, methyl ester
70	16.335	55277938	1.62	9,12,15-Octadecatrienoic acid (Z, Z, Z)
71	16.372	18051481	0.53	D-Alanine, N-(2,5-ditrifluoromethylbenzoyl)-, hexadecyl ester
72	16.499	11637972	0.34	Phenol,4,4'-(methylethylidene)bis-
73	16.529	1892645	0.06	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1-methyl-, [1S-(1 α ,4 $\alpha\alpha$,10 $\alpha\beta$)]-
74	16.801	7542645	0.22	[2-(1-butenyl)-2,2-dimethylcyclopropyl] acetonitrile
75	16.866	15484606	0.45	Benzeneacetamide, α -ethyl-
76	17.029	50938586	1.50	Benzene, 1,3,5-tributyl-
77	17.239	195813719	5.75	Azuleno[6,5-b]furan-2,5-dione,decahydro-4a,8-dimethyl-3-methylene-, [3aR-(3 $\alpha\alpha$,4 $\alpha\beta$,7 $\alpha\alpha$,8 β ,9 $\alpha\alpha$)]-
78	17.307	13097444	0.38	Hex-1-yne, 6-benzyloxy-
79	17.519	473361687	13.91	Benzene, 1-phenyl-4-(2,2-dicyanoethenyl)
80	17.556	7308815	0.21	Dicyclooctanopyridazine
81	17.669	81493286	2.39	Coumarine, 8-allyl-7-hydroxy-6-ethyl-4-methyl
82	17.793	173776292	5.11	Buta-1,3-diyne,1,4-bis(2-methoxycarbonylcyclopropyl)
83	17.821	7396321	0.22	Silane, dimethyl(2-naphthoxy) ethoxy-
84	17.942	37101465	1.09	3,3'-Difluoro-1,1'-biphenyl
85	17.995	4026095	0.12	3-Heptyne, 7-bromo-2,2-dimethyl-
86	18.031	2864819	0.08	2(5H)-Furanone, 4-(acetyloxy)-3,5-dimethyl
87	18.352	2490364	0.07	2-Ethylidenehydrazono-3methyl-4-chloro-2,3-dihydrobenzothiazole
88	18.418	5444559	0.16	Phthalic acid, 2,7-dimethyloct-7-en-5yn-4-yl-pentyl ester
89	18.512	3715125	0.11	4-Chloro-3-ethyl-1,3-benzothiazol-2(3H)-one
90	18.554	3540303	0.10	3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)- furanone
91	18.856	2363482	0.07	Bicyclo [3.1.0] hexane,4-methylene-1,6-diphenyl
92	19.068	2973834	0.09	Octadecane,3-ethyl-5-(2-ethylbutyl)-

Peak number	Retention time	Area time	Area %	Compound
93	19.591	1233693	0.04	5-p[-Anisyloxy]-6-methoxy-8-nitroquinoline
94	19.960	25919482	0.76	Bis(2'-hydroxy-3'-isopropylisobutyrophenonato) beryllium(ii)
95	20.080	33895435	1.00	1-Phenanthrenecarboxylic acid, tetradecahydro-1,4a,8-trimethyl-7-[2-[2-(methylamino) ethoxyl]-2-oxoethylidene]-9-oxo-, methyl ester, [1S-1 α ,4 α ,4b β ,7E,8 β ,8 α ,10a β]]
96	20.183	4779398	0.14	Pentatriacontane,13-docosenylidene
97	21.996	37593184	1.10	Vitamin E
98	24.444	5414430	0.16	Estra-1,3,5(10)-triene-16,17-dione, 3-[(trimethylsilyl)oxy]-, bis(O-methyloxime)

Table 4 shows a total of ninety-eight (98) biomolecular compounds identified in the methanol extract of *Laurus nobilis* leaf by GC-MS analysis. The five most abundant ($\geq 5\%$) compounds are Buta-1, 3-diyne, 1,4-bis(2-methoxycarbonyl

cyclopropyl) (5.11%), Azuleno[6,5-b]furan-2,5-dione, decahydro-4a,8-dimethyl-3-methylene-, 3aR-(3 α ,4 α ,7 α ,8 β ,9 α) (5.75%), n-Hexadecanoic acid (5.89%), phytol (7.57%) and Benzene, 1-phenyl-4-(2,2-dicyanoethenyl) (13.91%).

Table 5. Common bioactive compounds found in chloroform and methanol extract of *Laurus nobilis* leaf as identified by GC-MS analysis.

S/N	Compound	CELN Area %	MELN Area %	Pharmacological/Health Benefits
1	Eugenol	0.55	1.24	Anti-inflammatory [41] Antifungal [16], antioxidant, antibacterial, antimicrobial, anaesthetic, muscle relaxant [42, 43] and anticancer potential [44]
2	Benzene, 1,2-dimethoxy-4-(2-propenyl)	0.41	1.36	Antibacterial and antioxidant [43], antifungal [45]
3	Benzene, 1,2-dimethoxy-4-(1-propenyl)	0.11	0.11	Antibacterial, antifungal and antioxidant [16]
4	n-Hexadecanoic Acid (Palmitic Acid)	18.25	5.89	Anti-inflammatory [46], antimicrobial [47], antiplasmodial [48], antioxidant [49], wound healing [50]
5	7,10,13-Hexadecatrienoic Acid, Methyl Ester	12.73	1.35	Antioxidant, anti-inflammatory [51]
6	9,12,15-Octadecatrienoic Acid (Z, Z, Z) (Alpha-Linolenic Acid)	1.97	1.62	Anti-inflammatory, antimicrobial, antioxidant [52, 53]
7	Vitamin E	0.30	1.10	Antioxidant [54], immunomodulatory [55], skincare benefits [56, 57]
8	Isoaromadendrene Epoxide	1.89	0.17	Antimicrobial, anti-cancer [58, 59]
9	Azuleno[4,5-b]furan-2(3H)-one, 3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9-bis(methylene)	9.09	3.83	Antibacterial [60, 61], anticancer [62]
10	Caryophyllene (and Caryophyllene Oxide)	0.33	0.55	Analgesic and anticancer [63], anti-inflammatory and cytotoxic [64]

Table 5 shows ten (10) common bioactive compounds found in both CELN and MELN and their pharmacological benefits. The data show that n-Hexadecanoic Acid (Palmitic

acid) and 7,10,13-Hexadecatrienoic acid, Methyl Ester were the most abundant in both extracts.

4. Discussion

With the increasing demand for and widespread use of plant-derived herbal remedies, extensive research has been conducted to explore the medicinal potential of various plant species. Numerous active phytochemicals and bioactive compounds have been successfully extracted from different plant parts, including roots, leaves, and stems, contributing significantly to the discovery and development of therapeutic drugs for the treatment and management of a wide range of diseases. The present study provides a comprehensive qualitative and quantitative analysis of the bioactive constituents found in the chloroform and methanol extracts of *Laurus nobilis* (Bay leaf). This aromatic plant is commonly used as a culinary ingredient for flavour enhancement in Nigeria and many other regions of the world, is traditionally known for its potential health benefits.

Phytochemical analysis of the chloroform and methanol extracts of *Laurus nobilis* leaves revealed the presence of several bioactive compounds, including flavonoids, phenols, terpenoids, glycosides, steroids, saponins, alkaloids, and carbohydrates (Table 1). The results further indicate that the methanol extract (MELN) contained higher concentrations of phenols, tannins, and carbohydrates (Table 2) compared to the chloroform extract (CELN). These findings suggest that methanol may serve as a more effective solvent for extracting bioactive compounds from *Laurus nobilis* leaves, likely due to its polarity and enhanced ability to dissolve a wider range of phytochemicals. Carbohydrate is a key nutritional component of leaves of *Laurus nobilis* as our data show that carbohydrates as the highest phytochemical observed in both the chloroform (7.10mg/100g) and methanol leaf extracts (16.23mg/100g) methanol extracts). The high carbohydrate content in bay leaves suggests they could be a significant source of energy; however, they are typically used in small quantities as a culinary herb, primarily for flavouring rather than as a substantial energy source [65]. Other studies have shown that leaves of *Laurus nobilis* have significant quantities of carbohydrates alongside protein, calcium, magnesium, potassium, iron, phosphorus, copper, zinc, magnesium, manganese, and vitamins B12 and C [15, 65, 66].

GC-MS analysis of bioactive compounds identified key bioactive constituents in both CELN and MELN. The most abundant compounds ($\geq 5\%$) in CELN included Spiro(1,3,3-trimethylindoline)-2,5'-pyrrolidin-2-one (8.35%), 7,10,13-Hexadecatrienoic acid, methyl ester (12.75%), Azuleno[4,5-b]furan-2(3H)-one, 3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9-bis(methylene) (9.09%), and n-Hexadecanoic acid (18.25%) (Table 3). Similarly, the predominant compounds in MELN were Buta-1,3-diene, 1,4-bis(2-methoxycarbonylcyclopropyl) (5.11%), Azuleno[6,5-b]furan-2,5-dione, decahydro-4a,8-dimethyl-3-methylene-, 3aR-(3 α ,4 α ,7 α ,8 β ,9 α) (5.75%), n-Hexadecanoic acid (5.89%), phytol (7.57%), and Benzene, 1-phenyl-4-(2,2-dicyanoethenyl) (13.91%) (Table 4). Our findings also reveal that CELN and MELN

have ten (10) bioactive compounds in common with n-Hexadecanoic Acid (Palmitic acid) and 7,10,13-Hexadecatrienoic acid, Methyl Ester noted as the most abundant found in both extracts (Table 5). This wide range of phytochemical and bioactive compounds from *Laurus nobilis* gives it various pharmacological potency in the management and treatment of several disease conditions. Palmitic acid (n-Hexadecanoic acid) which is the common most abundant bioactive compound in both CELN and MELN is a saturated long-chain fatty acid found in both animal and plant sources (coconut oil, shea butter, cottonseed oil and sunflower oil). It possesses potent anti-inflammatory, anti-microbial, antiplasmodial, antioxidant and wound-healing activities [46-50]. Similarly, 0,13-Hexadecatrienoic acid, methyl ester, is a fatty acid ester derived from 10,13-hexadecatrienoic acid, a polyunsaturated fatty acid (PUFA) and found in various plant sources rapeseed, flaxseed, garden cress, borage and evening primrose. It is a potent antioxidant, antimicrobial and anti-inflammatory agent [51]. Another abundant compound common to both CELN and MELN is Azuleno[4,5-b]furan-2(3H)-one, 3a,4,6a,7,8,9,9a,9b-octahydro-6. It belongs to the azulene family and is found in other plants such as wormwood, chamomile and yarrow. They are noted for their muscle relaxant, anti-bacterial, anti-inflammatory, antioxidant and anti-cancer activities [60-62]. Eugenol is a naturally occurring phenolic compound commonly recognized for its aromatic properties found in scented plants like clove, basil, cinnamon and nutmeg. It has been shown to possess significant anti-inflammatory, anti-cancer, antioxidant, antimicrobial, and analgesic properties [16, 41-44]. Furthermore, CELN and MELN showed significant quantities of Vitamin E which is a group of fat-soluble compounds, including tocopherols and tocotrienols commonly extracted from oil-rich seeds such as coconut, corn, soybean and wheat germ oils. They are majorly known for their potent antioxidant properties as they play a crucial role in protecting cells and tissues from oxidative damage [54, 67]. They have also been shown to boost immune function [55] and have been extensively used in the formulation of skin care products for maintaining skin health [56, 57].

5. Conclusion

The present study underscores the rich phytochemical and bioactive profile of *Laurus nobilis* (bay leaf) extracts, with methanol emerging as a particularly effective solvent for extracting these compounds. The identified bioactive constituents have been extensively documented for their significant pharmacological properties, including anti-inflammatory, antimicrobial, antioxidant, and anticancer activities. These findings reinforce the potential of *Laurus nobilis* in disease management and highlight the need for comprehensive pharmacological exploration of its bioactive compounds to advance drug discovery efforts.

Abbreviations

GC–MS	Gas Chromatography-mass Spectrometry
CELN	Chloroform Extracts of <i>Laurus nobilis</i>
MELN	Methanol Extracts of <i>Laurus nobilis</i>

Author Contributions

Ngozi Nneka Offor: Conceptualization, Investigation, Project administration, Writing – original draft

Bruno Chukwuemeka Chinko: Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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