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Evaluation of Active Principles and Antibacterial Activity of *Chrysophyllium cainito* Leaf Extract Using Different Solvents

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Abstract

Chrysophyllum cainito, (*C. cainito*) commonly known as "Udara mbeke" in some places in Ebonyi State, Nigeria is a medicinal plant that has gained much attention due to its multiple benefits to human health. In this research, active principles and antibacterial activity of the leaf extracts were evaluated using selected clinically strain resistant organisms (*Escherichia coli*, *Staphylococcus aureus* and *Vibrio cholera*. Gas Chromatography-Mass Spectroscopy(GC-MS) results revealed a total of 21, 28, and 30 compounds in ethanol, n-hexane, and ethyl acetate extract respectively along side with their percentage total ion concentrations (%tic). The compound tentatively identified includes: Benzoic acid; 3-amino-3-methylbutyl ester (2.781%) with molecular weight 207, molecular formula $C_{12}H_{17}NO_2$, retention time (RT) 11.932 minutes, base peak at m/z of 137; Oxepine (11.789%) molecular weight 220**,** molecular formula $C_{15}H_{24}O$**,** RT 18.990 minutes**,** base peak at m/z 82 and1,2-Benzenedicarboxylic acid-bis(2-methylpropyl) ester (15.981%) with molecular weight of 278, molecular formula $C_{16}H_{22}O_4$, RT 11.561 minutes and base peak at m/z 149. The FTIR revealed absorption bands at 3608 cm^{-1}, 3257 cm^{-1} and 3246 cm^{-1}, from O−H stretching vibration of phenol, alcohol, and carboxylic acid. Alkanes (sp3 C−H stretching), were absorbed at 2926 cm^{-1}, 2922 cm^{-1}, and 2855 cm^{-1}. The *C. cainito* crude extracts exhibited strong antimicrobial property with zone of inhibition range of 16±1.50 to 36±2.00 mm. Ethyl Acetate extract has the highest Zone of Inhibition 36±2.00(mm) on *E. coli* followed by *V. cholerae* 30±4.00(mm) and *S. aureus* 28±2.00(mm). EtOH extracts shows 28±0.00 (mm) MIC on *E. coli* while *S. aureus* and *V. cholerae* have 16±1.50(mm) and 16±1.52(mm) respectively. N-Hexane extract was not effective at all against the three bacteria species.This finding suggested the potential application of *Chrysophyllumcainito* in the treatment and prevention of infectious diseases caused by pathogenic microorganisms.

Keywords: Antimicrobial, Bioactive, *Chrysophyllum cainito*,GC-MS.

Introduction

The value of plants are found in their bioactive constituents [1]. Phytochemicals are naturally occurring bioactive compounds found in various parts of plants [2], and the majority of spices, condiments, teas, and other beverages owe their individual properties to the pharmacologically active secondary metabolites that they contain. Due to their many uses, plant-derived compounds are highly sought after and frequently utilized in

conventional medicine as medications, food supplements, flavors, pharmaceutical intermediates, nutraceuticals, and other chemical entities [3, 4]. *Chrysophyllum cainito*, (Star Apple), is a tropical tree that belongs to the *Sapotaceae* family [5]. The plant has several medicinal uses and is reported to contain a variety of phytochemicals such as phenol, sterol, alkaloids, triterpenoids, and flavonoids [6]. Das *et al*., [7] showed that *C. cainito* leaf extract had anti-diabetic activity in an experimental rat. *C. cainito* is reported to reduce the blood glucose levels of the diabetic rabbits from 500 mg/dL to 140 mg/dL, over the course of six weeks [7]..Antioxidant qualities of the fruit and bark decoction used as cough suppressants or anti-tussive have been reported [5], while, Nweke *et al*. [8], evaluated *C. albidum* possible harm to the kidney of Wister rats. This study is targeted at evaluating the active principles and antibacterial activity of the *C. cainito* leaf extracts using selected clinically strain resistant organisms (*Escherichia coli*, *Staphylococcus aureus* and *Vibrio cholera*.

Materials and Method

Preparation of *Chrysophyllum cainito* leaf extracts

The leaves were collected within the community of Alex-Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria. It was properly identified, washed, air dried at room temperature and pulverized into powder using electric grinder. In 250 cm^3 beakers were introduced 10 g of the ground leaf sample, and immersed in 100mL of ethyl acetate, ethanol, or n-hexane for 72 hours respectively. The crude extracts were filtered using 110 mm Whatman filter paper and the filtrate was keep for analysis.

Determination of Bioactive principles by GC-MS

The identification of bioactive compounds in the *C. cainito* leaf extracts was performed using a gas chromatograph-mass spectrograph. The compounds were recognized by comparing their mass spectra and linear retention indices with those of the reference standard as described by Chukwu *et al.* [9].

FTIR analysis: For GC-FT IR analysis, a Bio-Radiation Digilab FTS-45A spectrometer coupled to a Bio-Radiation Tracer equipped with a liquid Nitrogen cooled narrow-band MCT detector and coupled to a HP 5890 series II gas chromatograph was used. The samples were eluted on a J and W DB-1 column 30 m × 0.25 mm (i.d.)/0.25 μm flick width with helium as stream gas (split injection mode). Deposition tip and transmission line were held above 200 °C. Absorbance spectra were recorded from 4000 to 700 cm^{-1} at a spectral resolution of 1cm^{-1} to identify the functional groups present. Also the fingerprint regions are then studied to positively identify the compounds**.**

Antimicrobial study

Clinical isolates of different microbial strains were collected from the Department of Microbiology, Alex Ekwueme Federal University, Ndufu Alike, Ebonyi State, Nigeria. The bacteria strains were negative (*Escherichia coli*and *Vibrio cholerae*) and Gram positive (*Staphylococcus aureus*). The

microbial strains were chosen based on their clinical and pharmacological relevance [10]. Antimicrobial screening was carried out using agar well diffusion method [11], and was carried out at the department of Microbiology, Alex Ekwueme Federal University, Ndufu Alike, Nigeria. The petri plates were prepared using sterile Muller–Hinton agar (MHA). The inocula of test cultures (106 CFU/mL) were streaked on to the condensed Muller Hinton agar in petri plates using a sterilized cotton swab, in order to ensure a uniform thick lawn or layer of growth, and allowed to dry for 15 minutes, a hole measuring 8 mm in diameter was made aseptically using a sterile cork borer. About 100µL concentration of *C. cainito*- crude stock solutions were prepared using 100% dimethyl sulfoxide (DMSO) as diluent 12]. The antimicrobial extract solutions were added at various concentrations in the hole. Agar plates were then incubated for 24 hours at 37°C. To gauge the antibacterial activity following incubation, the sensitivities of the microorganism species to the samples were determined by measuring the sizes of inhibitory zones 13].

Results and Discussion

Spectral data of Crude Ethanol, Ethyl acetate and n-Hexane Leaf Extract of *Chrysophyllum cainito*

These compounds were identified through mass spectrometry attached to a gas chromatograph and the results tabulated for the various samples. The active principles with their retention time (RT), base peak, percentage ion concentration (% tic), molecular formula and molecular weight (MW)), fragmentation pattern and biological activity in the crude leaf extracts are presented in Tables 1. A total of twenty-one (21) compounds where tentatively identified with their %tic in the crude extract of ethanol and they include: Oxepine (11.789%), Ketone- methyl-2-methyl-1-cyclohexene-1-yl-semicarbazone (1.197%), Others include: Benzoic acid-3-amino-3-methylbutyl ester (2.781%), (1-methylethyl)phenyl-Phosphine (2.001%), 1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-one (4.287%), (6R, 7R)-Bisabolone (1.449%) and Acetic acid, 3-(5,5-dimethyl-spiro[2.5]oct-4-yl)-1-methyl-propenyl ester (8.727 %).

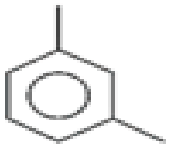
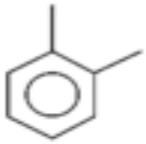
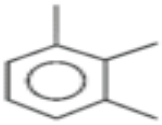
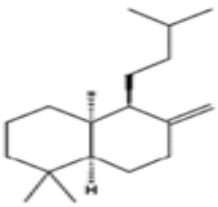
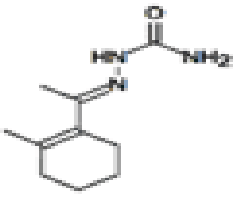
Table 1: Compounds detected from Crude Ethanol Leaf Extract of *Chrysophyllumcainito*

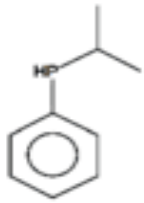
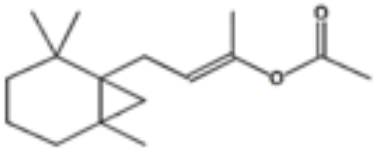
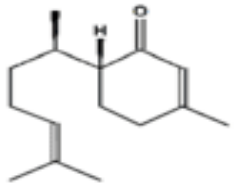
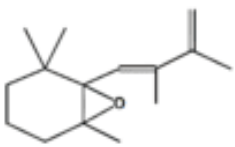
S/N	Name of compound	Molecular formular	Molecular weight	Base peak	Retention time(min)	TIC (%)
1	1,3-dimethyl-Benzene	C_8H_{10}	106	91	1.776	7.339
2	o-Xylene	C_8H_{10}	106	91	1.873	2.260
3	1,2,3-trimethyl-Benzene Mesitylene	C_9H_{12}	120	105	2.142	2.224
4	Decane,1,1'-oxybis-	$C_{20}H_{42}O$	298	57	6.080	2.493
5	(4aS, 5S,8aS) -5-Isopentyl-1,1, 4a - trimethyl - 6 – methylene decahydro naphthalene	$C_{19}H_{34}$	262	137	8.034	1.320

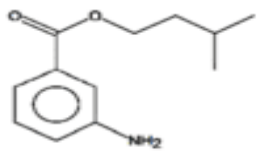
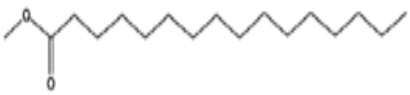
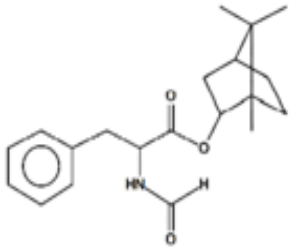
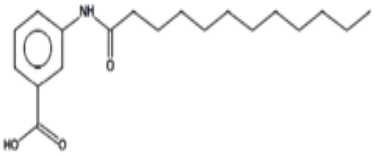
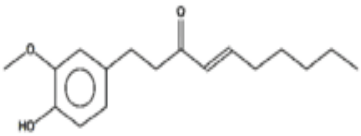
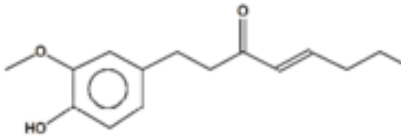
6	Methyl-2-methyl-1- cyclohexene -1-yl-semicarbazone	$C_{10}H_{17}N_3O$	195	137	8.229	1.197
7	Methyl stearate	$C_{19}H_{38}O_2$	298	74	8.371	1.673
8	1,12-dibromo-Dodecane	$C_{12}H_{24}Br_2$	326	55	8.491	1.009
9	1-methylethyl Phosphine	$C_9H_{13}P$	152	108	9.240	2.001
10	1-methyl-3- (2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-propenyl methyl ester	$C_{16}H_{26}O_2$	250	43	9.714	1.263
11	(6R, 7R)-Bisabolone	$C_{15}H_{24}O$	220	137	10.829	1.449
12	7-Oxabicyclo[7.1.0]heptane, 1-(2,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)-	$C_{15}H_{24}O$	220	137	11.189	6.688
13	Benzoic acid, 3-amino, 3-methylbutyl ester	$C_{12}H_{17}NO_2$	207	137	11.932	2.781
14	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	74	12.144	3.940
15	2-Formyl amino-3-phenylpropionic acid, 1,7,7-trimethylbicyclo [2.2.1]hept-2-yl-ester	$C_{20}H_{27}NO_3$	329	137	12.652	1.771
16	3-Lauramidobenzoic acid	$C_{19}H_{29}NO_3$	319	137	12.886	2.983
17	1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-one	$C_{17}H_{24}O_3$	276	137	13.647	4.287
18	1-(4-hydroxy-3-methoxyphenyl) oct-4-en-3-one	$C_{15}H_{20}O_3$	248	137	13.921	2.676
19	1-Benzenol,2-methoxy-4-[[[2-(4-hydroxyphenyl)ethyl]amino]methyl]-	$C_{16}H_{19}NO_3$	273	137	14.138	2.019
20	Acetic acid, 3-(5,5-dimethyl-spiro[2.5]oct-4-yl)-1-methyl-propenyl ester	$C_{16}H_{26}O_2$	250	137	17.584	8.727
21	(3R,5aS,9aR)-2,2,5a,9-Tetramethyl-3,4,5,5a,6,7-hexahydro-2H-3,9a-methanobenzo[b]oxepine	$C_{15}H_{24}O$	220	82	18.990	11.789

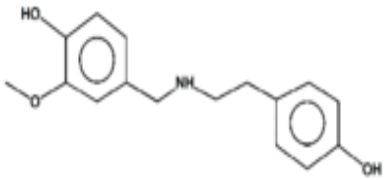
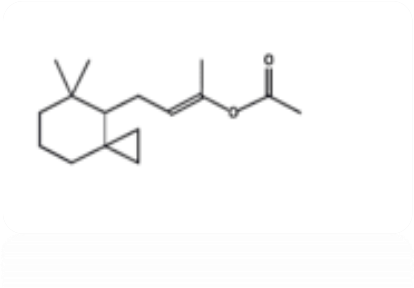
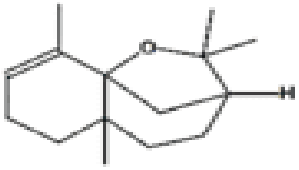
Compounds with antioxidants , anti-inflammatory and anti-microbial, properties in EtoH extract are 1,2,3- trimethyl- Benzene, (4aS, 5S,8aS) -5-Isopentyl-1,1, 4a -trimethyl - 6 – methylenedecahydronaphthalene, 1-methyl-3-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-propenyl methyl ester, Methylhexadecanoate, 1-Benzenol,2-methoxy-4-[[[2-(4-hydroxyphenyl) ethyl] amino] methyl]- and (6R, 7R)-Bisabolone. Those reported to have antiviral and anti cancer properties include: 1-(4-hydroxy-3-methoxyphenyl) oct-4-en-3-one, Benzoic acid-3-amino-3-methylbutyl ester 7-Oxabicyclo [7.1.0] heptane, 1-(2,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)- and Methyl-2-methyl-1- cyclohexene -1-yl- semi carbazone (Table 2).

Table 2: Structure and biological Activity of Compounds detected in Crude Ethanol Leaf Extracts of *C. cainito*

S/N	Name of compound	Structure	Activity
1	1,3-dimethyl- Benzene		Solvent, couplers of medicines, spices, dye
2	o-Xylene		phthalic anhydride, vitamin and pharmaceutical syntheses, dyes, insecticides, motor fuels
3	1,2,3- trimethyl- Benzene		Antioxidants, dye, pharmaceuticals
4	1,1-oxybis-Decane		*Antimicrobial, Cosmetic agent*
5	(4aS, 5S,8aS) -5-Isopentyl-1,1, 4a -trimethyl - 6 – methylenedecahydronaphthalene		Antioxidant, anti-inflammatory, anti-microbial
6	Methyl-2-methyl-1-cyclohexene -1-yl- semi carbazone		Catalysts, antifertility, antiviral, anti-infective, antineoplastic and enzymatic agents

7	Methyl stearate		Nonionic Surfactant, Emulsifier and stabilizer
8	1,12-dibromo-dodecane		solvent, distillation chaser, and scintillator
9	1-methylethyl Phosphine		Organophosphorus , fumigant, flame retardants
10	1-methyl-3- (2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-propenyl methyl ester		Antiseptic against pseudomonas, enterococci, streptococci, staphylococci. Cervical cancer screening and for the treatment of infections.
11	(6R, 7R)-Bisabolone		Antioxidant, repair the skin barrier and speed up the healing time of sunburn
12	7-Oxabicyclo [7.1.0] heptane, 1-(2,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)-		Antitumor inhibitory activity

13	Benzoic acid-3-amino-3-methylbutyl ester		Antiviral, antibacterial and antiulcer properties
14	Methylhexadecanoate		strong antioxidant and radical-scavenging potential, as well as inhibition of lipid peroxidation
15	2-Formyl amino-3-phenylpropionic acid, 1,7,7-trimethylbicyclo [2.2.1] hept-2-yl-ester		Antimalaria, renin-inhibiting properties
16	3-Lauramidobenzoic acid		Antifungal, anti-inflammation property, skin irritation ointment and drug preservatives
17	1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-one		Anti-inflammatory, antidiabetic, antilipolytic, antidiarrhoeic properties
18	1-(4-hydroxy-3-methoxyphenyl) oct-4-en-3-one		Free radical scavengers

19	1-Benzenol,2-methoxy-4-[[[2-(4-hydroxyphenyl) ethyl] amino] methyl]-		Antioxidant, Antiemetic used to treat postoperative nausea and vomiting
20	Acetic acid, 3-(5,5-dimethyl-spiro [2.5] oct-4-yl)-1-methyl-propenyl ester		Building blocks
21	(3R,5aS,9aR)-2,2,5a,9-Tetramethyl-3,4,5,5a,6,7-hexahydro-2H-3,9a-methanobenzo[b]oxepine		Treatment of epilepsy (seizures). Antiepileptics or anti-convulsant

Tables 3 – 4 depict the spectral data of crude ethyl acetate leaf extract of *C. cainito*. A total of thirty (30) compounds where tentatively identified which include: 1,3-dimethyl-benzene (25.394 %), 2,4-Di-tert-butylphenol (3.026%), Hexadecanoic acid, methyl ester (1.527%), 9-[2-Hydroxyhexyl] hypoxanthine (0.881%). Others include: Sulfurous acid, 2-propyl tetradecyl ester (1.576%), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (15.981%) and Tetratetracontane (4.64%) . Those identified as anti cancer, antibacterial and ant inflammatoryin the ethyl acetate extract include: Decane, 1,2,4-trimethyl-benzene, 2,4-Di-tert-butylphenol, Hexadecane, 3-Oxatricyclo [4.1.1.0(2,4)] octane, 2,7,7-trimethyl-,Hentriacontane, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, Hexadecanoic acid, methyl ester, 1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one. (Shogoal), Spiro [4.5] decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl- and Tetratetracontane (Table 4).

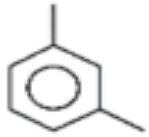
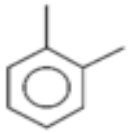
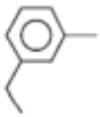
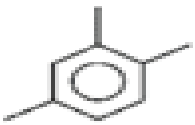
Spectral Data of Crude Ethyl Acetate Leaf Extract of *Chrysophyllumcainito*

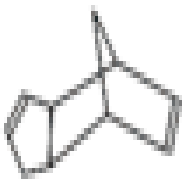
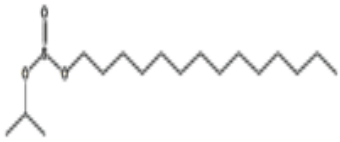
Table 3: Compounds detected from Crude Ethyl Acetate Leaf Extract of *C. cainito*

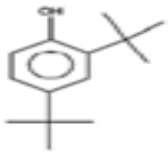
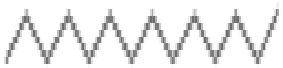
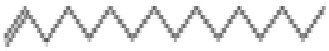
S/N	Name of compound	Molecular formular	Molecular weight	Base peak	RT (min)	TIC (%)
1	1,3-dimethyl-benzene	C_8H_{10}	106	91	1.776	25.394
2	o-Xylene	C_8H_{10}	106	91	1.867	2.707
3	1-ethyl-3-methyl-benzene	C_9H_{12}	120	105	2.148	0.894
4	Decane	$C_{10}H_{22}$	142	57	2.262	1.565
5	1,2,4-trimethyl-benzene	C_9H_{12}	120	105	2.336	2.369
6	Dicyclopentadiene	$C_{10}H_{12}$	132	66	2.599	1.102
7	3a,4,5,6,7,7a-Hexahydro-4,7-methanoindene	$C_{10}H_{14}$	134	66	2.931	2.997
8	4,7-Methano-1H-indene, octahydro-	$C_{10}H_{16}$	136	95	3.153	1.294
9	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, (1R-endo)-	$C_{10}H_{18}O$	154	81	3.542	0.849
10	Sulfurous acid, 2-propyl tetradecyl ester	$C_{17}H_{36}O_3S$	320	43	4.611	1.576
11	1-Tetradecene	$C_{14}H_{28}$	196	43	6.000	1.454
12	Dotriacontane, 1-iodo-	$C_{32}H_{65}I$	576	57	7.120	3.641
13	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	191	7.497	3.026
14	Tetradecane	$C_{14}H_{30}$	198	57	7.674	1.015
15	Cetene	$C_{16}H_{32}$	224	43	8.417	2.013
16	Hexadecane	$C_{16}H_{34}$	226	57	8.492	0.622
17	Nonyl Pentyl Sulphonic acid	$C_{14}H_{30}O_3S$	278	71	9.029	0.843
18	3- Oxatricyclo [4.1.1.0(2,4)] octane, 2,7,7-trimethyl-	$C_{10}H_{16}O$	152	67	9.229	1.095
19	Hentriacontane	$C_{31}H_{64}$	436	57	9.617	3.137
20	1-Octadecene	$C_{16}H_{36}$	252	43	10.709	1.650
21	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	149	11.561	15.981
22	(1R,3aR,5aR,9aS)-1,4,4,7-Tetramethyl-1,2,3,3a,4,5a,8,9-octahydrocyclopenta[c]benzofuran	$C_{15}H_{24}O$	220	205	12.092	1.137
23	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	74	12.144	1.527
24	9-[2-Hydroxyhexyl] hypoxanthine	$C_{11}H_{16}N_4O_2$	236	149	12.567	0.881
25	Heptacos-1-ene	$C_{27}H_{54}$	378	57	12.824	1.401
26	1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one	$C_{17}H_{24}O_3$	276	137	13.298	1.120

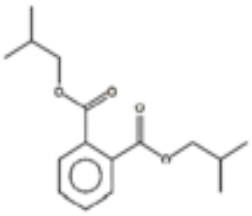
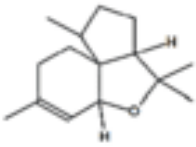
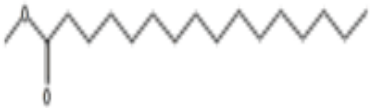
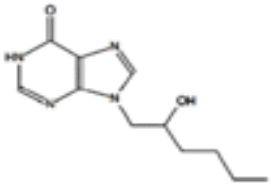
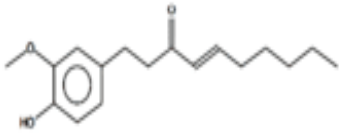
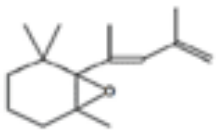
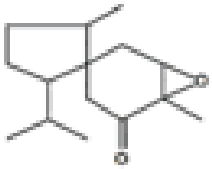
27	7-Oxabicyclo [4.1.0] heptane, 1-(1,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)-	$C_{15}H_{24}O$	220	137	13.692	1.600
28	Spiro [4.5] decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	$C_{15}H_{24}O_2$	236	43	13.921	1.340
29	Tetratetracontane	$C_{44}H_{90}$	618	57	14.014	4.68
30	Heptadecanoic acid, 10-methyl-, methyl ester	$C_{19}H_{38}O_2$	298	74	14.138	0.94

Table 4: Structures and biological activities of Compounds identified from Crude Ethyl Acetate Leaf Extracts

S/N	Name of compound	Structure	Activity
1	1,3- dimethyl-benzene		Solvent, couplers of medicines, spices, dye
2	o-Xylene		Tissue processing, staining and cover slipping in the histology laboratory.
3	1-ethyl-3-methyl-benzene		Natural product
4	Decane		Antitumor agent, Jet fuel, solvent
5	1,2,4-trimethyl-benzene		Gasoline additives, pharmaceuticals, dyes Antioxidants and solvent

6	Dicyclopentadiene		Fuel additives, Pesticides, paints, inks and varnishes production
7	3a,4,5,6,7,7a-Hexahydro-4,7-methanoindene		Organo metallic compound. Pesticides, monosodium glutamate and pet resins
8	4,7-Methano-1H-indene, octahydro-		Fragrance agent
9	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, (1R-endo)-		Food additive, perfuming agent or fragrance in cosmetics.
10	Sulfurous acid, 2-propyl tetradecyl ester		Antiseptic, Osmotic laxative to clean colon before colonoscopy. Skin ointment
11	1-Tetradecene		Solvent in perfumes, medicine, dyes oils and resins
12	Dotriacontane, 1-iodo-		Glazing agent and texturizer for chewing gum

13	2,4-Di-tert-butylphenol		Antibacterial, Antifungal and Anticancer
14	Tetradecane		Solvent used for analyte with a low boiling point.
15	Cetene		To maintain the health of skin, cartilage, teeth, bone, and blood vessels
16	Hexadecane		Antibacterial, Antioxidant.
17	Nonyl Pentyl Sulphonic acid		Antiseptic, Osmotic laxative to clean colon before colonoscopy. Skin ointment
18	3-Oxatricyclo [4.1.1.0(2,4)] octane, 2,7,7-trimethyl-		Antimicrobial and antifungal properties. Use in treatment of various infections
19	Hentriacontane		Anti-inflammatory, antitumor and antimicrobial activities.
20	1-Octadecene		A reactive chemical that is used as an Antimicrobial agent.

21	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester		Anticancer and immunomodulatory-cell stimulant
22	(1R,3aR,5aR,9aS)-1,4,4,7-Tetramethyl-1,2,3,3a,4,5a,8,9-octahydrocyclopenta[c]benzofuran		Antitumor, antibacterial, antioxidant, and antiviral activities.
23	Hexadecanoic acid, methyl ester		Anti-inflamatory, Strong anti-oxidant, Antibacterial.
24	9-[2-Hydroxyhexyl] hypoxanthine		Additive in cells, bacteria and parasite cultures and nitrogen source.
25	Heptacos-1-ene		Natural substance
26	1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one. (Shogoal)		Anticancer, Antioxidant, Antimicrobial, Anti-allergic, Anti-inflammatory
27	7-Oxabicyclo [4.1.0] heptane, 1-(1,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)-		Antitumor agent, Agrochemical
28	Spiro [4.5] decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-		Antioxidant, Antimicrobial, Antiobesity, Antidiabetic Cardiovascular protective.

29	Tetratetracontane		Antioxidant, Cytoprotective activity
30	Heptadecanoic acid, 10-methyl-, methyl ester		Quantification of fatty acid methyl ester in human plasma

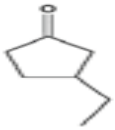
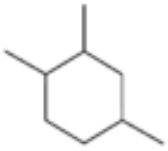
Tables 5-6 show the spectral data from crude n-Hexane leaf extracts of *C. cainito*. A total of twenty-eight (28) compounds where tentatively identified with their %tic and they include:3-Ethylcyclopentanone (14.291%), (E) -1-butoxy-2-Hexene (7.307%),. Yangambin (1.08 %) and Bis(2-ethylhexyl) phthalate (4.61%). However, those compounds identified as anti cancer, antioxidant, antibacterial and ant inflammatory in the ethyl acetate extract include: Decahydro-Naphthalene (Decalin), Undecane, 2-Methyl-3-trans-propenylpyrazine,trans-4-Methyl-decahydronaphthalene, Dodecane, Benzoic acid-3-amino-, 3-methylbutyl ester, 1-Benzenol,2-methoxy-4-[[[2-(4-hydroxyphenyl) ethyl] amino] methyl]-, 2-hydroxy-N-(1,2,2-trimethylpropyl)-Benzamide**,** 3-Iodomethyl-3,6,6-trimethyl-cyclohexene, Yangambin, 1,3-bis(3-phenoxyphenoxy)-benzene and 1,7-bis(3,4-Dimethoxyphenyl) heptane-3,5-diyl diacetate (Table 6).

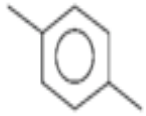
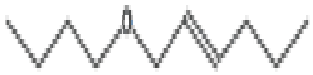
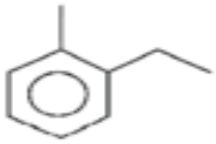
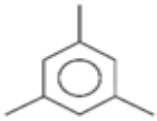
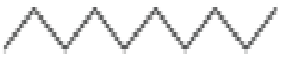
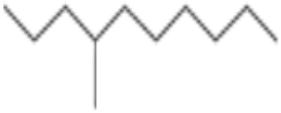
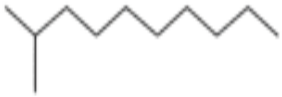
Table 5: Components Detected from Crude n-Hexane Leaf Extract of *C. cainito*

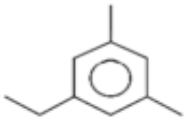
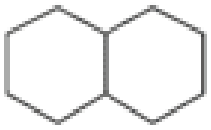
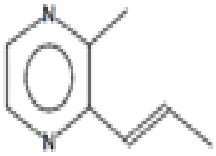
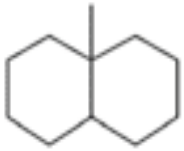
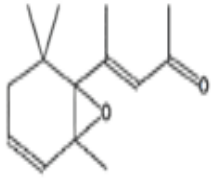
S/N	Name of compound	Molecular formula	Molecular weight	Base peak	Retention Time (min)	TIC (%)
1	3-Ethylcyclopentanone	$C_7H_{12}O$	112	83	1.673	14.291
2	1,2,4-trimethyl-, (1α,2β,4β)-cyclohexane	C_9H_{18}	120	69	1.725	2.014
3	p-Xylene	C_8H_{10}	106	91	1.770	3.282
4	Nonane	C_9H_{20}	128	43	1.810	10.846
5	2-Hexene, 1-butoxy-, (E)-	$C_{10}H_{20}O$	156	57	1.976	7.307
6	1-ethyl-2-methyl-benzene	C_9H_{12}	120	105	2.136	1.281
7	Mesitylene	C_9H_{12}	120	105	2.182	1.116
8	Decane	$C_{10}H_{22}$	142	57	2.256	5.560
9	4-methyl-decane,	$C_{11}H_{24}$	156	43	2.382	1.036
10	2-methyl-decane,	$C_{11}H24$	156	57	2.656	0.79

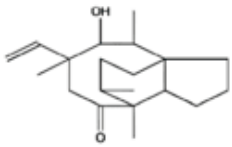
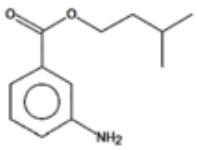
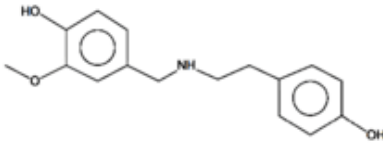
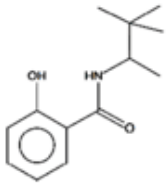
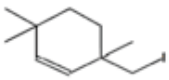
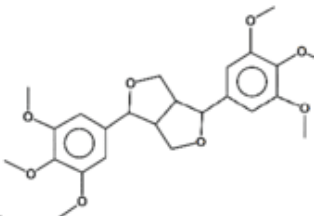
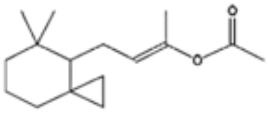
11	1-ethyl-3,5-dimethyl- benzene	$C_{10}H_{14}$	134	119	2.708	0.78
12	Naphthalene, decahydro-	$C_{10}H_{18}$	138	138	2.833	0.86
13	Undecane	$C_{11}H_{24}$	156	57	2.931	2.68
14	2-Methyl-3-trans-propenylpyrazine	$C_8H_{10}N_2$	134	119	3.262	0.58
15	Trans-4-Methyl-decahydronaphthalene	$C_{11}H_{20}$	152	95	3.416	0.60
16	Dodecane	$C_{12}H_{26}$	170	57	3.828	0.96
17	4-(2,2,6-Trimethyl-7-oxabicyclo[4.1.0]hept-4-en-1-yl)pent-3-en-2-one	$C_{14}H_{20}O_2$	220	137	11.132	0.70
18	Tetradecane, 1-bromo-	$C_{14}H_{29}Br$	276	57	11.624	0.83
19	Tricyclo[5.4.3.0(1,8)]tetradecan-6-one, 4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl	$C_{20}H_{32}O_2$	304	149	12.527	0.56
20	Benzoic acid-3-amino-3-methylbutyl ester	$C_{12}H_{17}NO_2$	207	137	13.430	0.77
21	1-Benzenol,2-methoxy-4-[[[2-(4-hydroxyphenyl)ethyl]amino]methyl]-	$C_{16}H_{19}NO_3$	273	137	14.018	0.86
22	2-hydroxy-N-(1,2,2-trimethylpropyl)-benzamide	$C_{13}H_{19}NO_2$	221	121	14.693	1.18
23	3-Iodomethyl-3,6,6-trimethyl-cyclohexene	$C_{10}H_{17}I$	264	137	18.190	3.71
24	Yangambin	$C_{24}H_{30}O_8$	446	446	16.081	1.08
25	1,3-bis(3-phenoxyphenoxy)-benzene	$C_{30}H_{22}O_4$	446	446	16.167	4.27
26	Acetic acid, 3-(5,5-dimethyl-spiro [2.5] oct-4-yl)-1-methyl-propenyl ester	$C_{16}H_{26}O_2$	250	137	17.093	4.48
27	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	149	17.762	4.61
28	1,7-bis (3,4Dimethoxyphenyl) heptane-3,5-diyl diacetate	$C_{27}H_{36}O_8$	488	151	18.956	2.41

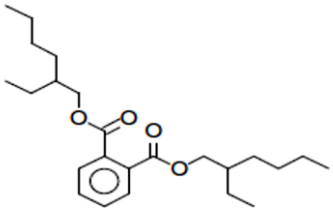
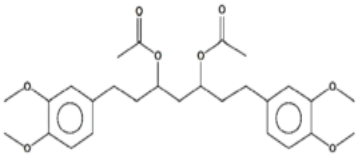
Table 6: Major Bioactive Compounds present in crude n-Hexane Leaf Extract of *C. cainito*

S/N	Name of Compound	Molecular structure	Activity
1	3-Ethylcyclopentanone		Pharmaceutical reagent, Pesticides, perfume additives.
2	1,2,4-trimethyl-, (1α,2β,4β)-cyclohexane		Manufacture of pharmaceutical

3	p-Xylene		Processing and staining tissues in histology
4	Nonane		Jet fuel, Solvent, Biodegradable detergent.
5	2-Hexene, 1-butoxy-, (E)-		Perfume fragrance, comonomer in production of HDPE
6	1-ethyl-2-methyl-benzene		To predict novel lead compounds for drug discovery that depends on the quality of the docking scoring function.
7	Mesitylene		Used as a specialty solvent in the laboratory.
8	Decane		Organic solvent, Jet-fuel
9	4-methyl-decane		Antifungal activity
10	2-methyl-decane		Drug manufacturing agent.

11	1-ethyl-3,5-dimethyl-benzene		As a benzene derivative, analgesic and antipyretic properties
12	Decahydro-Naphthalene (Decalin)		Anti inflammatory
13	Undecane		Anti-allergic, anti-inflammatory, sex attractant
14	2-Methyl-3-trans-propenylpyrazine		Antituberculosis, antitumor
15	trans-4-Methyl-decahydronaphthalene		Antifungal antibacterial, anticancer, immunosuppressive activity.
16	Dodecane		Antibacterial and antifungal activity.
17	4-(2,2,6-Trimethyl-7-oxabicyclo [4.1.0] hept-4-en-1-yl) pent-3-en-2-one		Monoterpene used as a flavouring agent and antiseptic
18	1-bromo-Tetradecane		Synthesis of medicine and fine chemicals

19	Tricyclo[5.4.3.0(1,8)]tetradecan-6-one, 4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl		As pleuromutilin, it is used as antibacterial drug that inhibits protein synthesis in bacteria
20	Benzoic acid-3-amino-, 3-methylbutyl ester		Antibacterial, Antifungal, Insect attractant.
21	1-Benzenol,2-methoxy-4-[[[2-(4-hydroxyphenyl) ethyl] amino] methyl]-		Antioxidant, Antiemetic used to treat postoperative nausea and vomiting
22	2-hydroxy-N-(1,2,2-trimethylpropyl)- Benzamide		Antimicrobial, analgesic, anticancer, carbonic anhydrase and cholinesterase inhibitory activities
23	3-Iodomethyl-3,6,6-trimethyl-cyclohexene		Antimicrobial activity
24	Yangambin		Antiallergic activity against β-hexosaminidase release Anti-inflammatory.
25	1,3-bis(3-phenoxyphenoxy)-benzene		Antimicrobial Natural product
26	Acetic acid, 3-(5,5-dimethyl-spiro [2.5] oct-4-yl)-1-methyl-propenyl ester		As an eardrop it is used to treat infection of the ear canal

27	Bis(2-ethylhexyl) phthalate		Antimutagenic activity. Potential chemoprotective compound, Antifungal, Antioxidant, Antitumor
28	1,7-bis(3,4-Dimethoxyphenyl) heptane-3,5-diyl diacetate		As a diarylheptanoid, it possesses anti-ulcer, anti-bacterial, anti-cathartic, antidiabetic, hepato-protective, cholesterol level lowering

The FTIR spectra of *C. cainito* extracts from the various solvents are shown in Table 7-9. It portrayed a variety of absorption bands ranging from 3608cm^{-1} to 685cm^{-1}, which pointed to the presence of various functional groups. The absorption bands at 3608 cm^{-1}, 3257 cm^{-1} and 3246 cm^{-1}, resulted from O−H stretching vibration of phenol, alcohol, and carboxylic acid. Alkanes (sp3 C−H stretching), were absorbed at 2926 cm^{-1}, 2922 cm^{-1}, and 2855 cm^{-1}. Similar findings were reported by Isaac *et al.* [14]; and Kero *et al*. [15]. N=C=S stretching band were observed at 2098 cm^{-1} and indicated the existence of isothiocyanate. While the bands at 2117 cm^{-1} and 2113 cm^{-1}, corresponded to C≡C stretch of alkyne compounds.

The band at 1986cm^{-1} corresponded to C=C=C stretching (Allene), the band at 1997 cm^{-1} represented C−O stretching from phenol. The C=O stretching vibration, which was either from acyl chloride, ketone, aldehyde, carboxylic acid, or ester, is what caused the bands at 1882 cm^{-1} and 1729 cm^{-1}. N−H bending vibration from amine was shown by the bands at 1587 cm^{-1}, whereas C−H bending vibration from proteins was indicated by the bands at 1386 cm^{-1} and 1371 cm^{-1}. This was consistent with the study by Prakash *et al*. [16]. The C=C stretching molecule caused the band at 1606 cm^{-1} to vibrate. The compounds detected by GCMS are compatible with the functional groups depicted by FTIR analysis of *C. cainito* leaf extracts of different solvents.

Tables 7-9 depict Fourier Transform Infrared (FT-IR) Spectrum of the *C. cainito* leaf in EtOH , ethyl acetate and n-hexane extract with diagonistic absorption bands indicated below:

Table 7: FT-IR spectral peak values and functional groups obtained from EtOH extract

S/N	Wavenumber (cm^{-1})	Functional groups
1	3246	O-H stretch (carboxylic acid)
2	2926	C-H stretch (sp3)
3	2117	C≡C stretch
4	1729	C=O stretch (ester)
5	1587	N-H bend (Amine)
6	1386	C-H bend
7	1248	O=C-O-C stretch (acetate/ aromatic esters)
8	1025	=C-O-C sym. (Ethers)
9	816	C-H bend

Table 8: FT-IR spectral peak values and functional groups obtained from ethyl acetate extract

S/N	Wavenumber (cm-1)	Functional group
1	3257	O-H stretch
2	2922	C-H stretch
3	2855	C-H stretch Sp3
4	2113	C≡C stretch
5	1997	C-O stretch (Ar-O-H)
6	1606	C=C Aromatic ring stretch
7	1341	C-F stretch
8	1025	=C-O-C sym. (R-O-R, Ethers)
9	823	C-Cl stretch
10	685	C-Br stretch

Table 9: FT-IR spectral peak values and functional groups obtained from n-Hexane extract

S/N	Wavenumber (cm-1)	Functional group
1	3608	O-H stretch
2	2098	N=C=S stretch (Isothiocyanate)
3	1986	C=C=C stretch (Allene)
4	1882	C=O stretch (Acyl chloride)
5	1587	N-H bend (Amine)
6	1371	C-H bend
7	1028	=C-O-C sym. (Ethers)
8	790	=C-H bend

Antibacterial Analysis of Crude extracts of *C. cainito* leaf from EtOH, n-hexane and ethyl acetate

The Crude extracts of *C. cainito* leaf from EtOH, n-hexane and ethyl acetate solvents were tested for antibacterial activity against both Gram's positive (*Staphylococcus aureus*) and Gram's negative (*Escherichia coli* and *Vibrio cholerae*) bacteria (Table 10). Ethyl Acetate has the highest Zone of Inhibition 36±2.00(mm) on *E. coli* followed by *V. cholerae* 30±4.00(mm) and *S. aureus* 28±2.00(mm). Ethanol extracts shows 28±0.00 (mm) MIC on *E. coli* while *S. aureus* and *V. cholerae* have 16±1.50(mm) and 16±1.52(mm) respectively. N-Hexane extract was not effective at all against the three bacteria species. This suggested that the extracts in n-Hexane could not stop the growth of bacteria and may not be effective or good solvent for the study as it was highly volatile. EtOH extract with superscript 'a' had a statistically significant different effect compared to Ethyl acetate with superscript 'b' with respect to their zone of inhibitions while EtOH extract with superscript 'c' had a significantly different effect with Ethyl acetate extract with superscript 'd' for *E. coli*,*S. aureus* and *Vibrio cholerae* respectively.

Table 10: Result for the Minimum Inhibitory Concentration (MIC) of the crude extracts of *C. cainito* Leaf

Extracts code/name	Zone of Inhibition against *E.coli* (mm)	Zone of inhibition against *S. aureus* (mm)	Zone of inhibition against *Vibrio cholerae*(mm)	Inference
EtOH extract (leaf)	28 ± 0.00^{a}	16 ± 1.50^{c}	16 ± 1.52^{a}	Effective against all
n-Hexane extract (leaf)	NE	NE	NE	Not effective at all
Ethyl acetate (leaf)	36 ± 2.00^{b}	28 ± 2.00^{d}	30 ± 4.00^{c}	Very effective against the three species of bacteria.
P value	0.001	0.001	0.001	

Values with Different Superscripts are Significantly Different ($P<0.05$)
NE = Not Effective

Fig. 1: (a) Plates Showing Zone of Inhibitions from Crude C. *cainito* Plant Extracts *Vibrio* (n-Hexane) *S. aureus* (EtOH) *V. cholerae E. coli* (ethyl acetate)

Conclusion

The active principles identified in the various fractions with their percentage ion concentration the plant extract include: Benzoic acid; 3-amino-3-methylbutyl ester (2.781%) , Oxepine (11.789%) , 1,2,3- trimethyl- Benzene, (4aS, 5S,8aS) -5-Isopentyl-1,1, 4a -trimethyl - 6 – methylenedecahydronaphthalene (1.320), (6R, 7R)-Bisabolone (1.449), Benzoic acid-3-amino-3-methylbutyl ester in the ethanol extract. Most of which are reported as Antioxidants, *Antimicrobial,* inflammatory and antiviral agent. 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester and 1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one were reported as anti cancer, antioxidant and antibacterial agent was identified in the ethyl acetate fraction, while 3-Iodomethyl-3,6,6-trimethyl-cyclohexene and Yangambin from the n-hexane fraction are known to have antimicrobial, antiallergic and anti-inflammatory activity respectively. The antibacterial activity of the leaf extracts on clinically strain resistant organisms (*Escherichia coli*, *Staphylococcus aureus* and *Vibrio cholera* were promising.

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