

Comparative study of the Chemical Composition and Antimicrobial Activity of Essential oils obtained From Seeds and Leaves of Star Apple (Chrysophyllum cainito L.)

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Abstract

In this study, the essential oils of seeds and leaves from Star Apple (*Chrysophyllum cainito* L.) were profiled and screened for their antimicrobial potential. The volatile oils were isolated by means of hydro-distillation process using a Clevenger-type apparatus. The oils were analysed by gas chromatography coupled with mass spectrometry (GC-MS). Antimicrobial activity of the isolated essential oils was investigated by agar well diffusion method against seven bacterial isolates and two fungal isolates. A total of thirty-one chemical constituents were identified in essential oil of leaves with β -ocimene (12.37%), TMTT 1,3,7,11-Tridecatetraene (9.73%), α -farnesene (9.19%) and cyclofenchene (7.16%) as major components. Twenty-eight compounds were characterised in the seeds oil with citral (36.60%), Geranial, (28.50%) and (E)-3,7-Dimethylocta-3,6-dienal (10.82%) as main constituents. The two oils contained beta and alpha ocimene, cyclofenchene and pelargonaldehyde in common. The lowest MIC observed with essential oil of the leaves was 0.781251 mg/mL for *Staphylococcus aureus* and 1.625 mg/mL for *Escherichia coli*. The lowest MIC recorded for essential oil of the seeds was 12.5 mg/mL for *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. Essential oil obtained from leaves exhibited higher antimicrobial and antifungal activities against all tested organisms than essential oil of seeds. The good to moderate antimicrobial results in this study has further justified the ethnomedicinal use of *C. cainito* in the treatment of inflammation caused by pneumonia and laryngitis microbes.

Journal highlights

- This is the first report on the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and the minimal fungicidal concentration (MFC) of *C. cainito* leaf and seed oils.
- The current research report for the first time a comparative analysis of the chemical constituents of *C. cainito* leaf and seed oils.
- Also, this research reports the biological significance of each of the dominant constituents of *C. cainito* leaf and seed oils.

1. Introduction

Chrysophyllum cainito L. (*C. cainito*) is a tree plant found in tropical regions, it belongs to Sapotaceae family. *C. cainito* is a fruit-bearing tree grown for its ornamental purpose. The skin is rich in latex; though the skin and the rind are not edible. The seed is flat, hard and light brown in colour [1].

There are three different species of *C. cainito* depending on its origin such as star apple, cainito, and caimito [2–3]. There are about 4 types of *C. cainito* fruit locally grown in Indonesia [4].

The centre of the seed of *C. cainito* has the appearance of an asterisk when cut transversely, hence it is called “star apple.” *C. cainito* tree species is commonly found in Argentina, Mexico, Peru, at the Pacific side of Guatemala, and Vietnam, Malaysia, China, India, and other countries with low altitudes [5, 3]. Since ages, *C. cainito* has been used in tradomedicinal healthcare system. The bioactivity of *C. cainito* has been reported recently, which included antioxidant potential [6], antidiabetic activity [7–8], anti-inflammatory potential [9], anticancer activities [10], and antihypertensive property [11], Preliminary phytochemical screening of *Chrysophyllum cainito* leaf extract indicated the presence of saponins, sterols, flavonoids, glycosides, tannins [12–13].

Pino *et al.* [22] and Oranusi *et al.* [13] reported on the chemical composition of *Chrysophyllum cainito* extracts, their essential oils (EOs) and their antimicrobial activities on bacteria. However, comparative studies on the chemical compositions and antimicrobial activities of the essential oils of the leaves and seeds of *Chrysophyllum cainito* is limited. Therefore, comparative evaluation of the seeds and leaves EOs is necessary. This study therefore was aimed to investigate comparatively the chemical compositions of the EOs of the seeds and leaves of *Chrysophyllum cainito*, and to screen the oils for their antimicrobial potential against clinical isolates of selected bacteria and fungi in order to validate their ethnomedicinal use for treatment of inflammation caused by pneumonia and laryngitis [14].

2. MATERIALS AND METHODS

2.1 Sample Collection Extraction of Plant Materials

The *Chrysophyllum cainito* leaf and seed samples were collected at Ajayi Crowther University, Oyo, Oyo-State, Nigeria, in February, 2022. The seed and leaf samples were identified at the Herbarium section of Botany Department, University of Ibadan. Air dried samples were pulverised. Batches of 500 g plant materials were subjected to hydrodistillation process for 3 hr using a Clevenger-type apparatus designed according to British Pharmacopeia with slight modifications. The resulting EOs were collected in vial bottles, weighed and refrigerated at 4 °C prior to analysis.

2.2 GC-MS analysis of essential oil

The analysis of the EOs to determine the chemical composition of the oils was done with the use of GC-MS. The analysis was carried out using an 8869A gas chromatograph coupled to a 5979C inert mass spectrometer on an electron impact source (Agilent Technologies). The stationary phase was HP-5 capillary sandwiched with 5% of Phenyl Methyl Siloxane (25 m length $\times$ 0.43 mm diameter $\times$ 0.45 μ m film thickness), being an Agilent Technologies.

The carrier gas was Argon used at a constant flow rate of 1.658 mL/min, an initial nominal pressure of 1.9863 psi and at an average rate velocity of 55 cm/s. About one microliter of the samples was injected with 60:1 Split mode at an injection temperature of 300°C. Purge flow was 21.5 mL/min at 0.50 min with a total gas flow rate of 23.355 mL/min. The oven was initially programmed at 38°C (for 1 min), then ramped at 10°C/min to 280°C (3 min). Run time was 30.25 min with a 3 min solvent delay.

The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 280°C. Scanning of possible compounds was from m/z 50 to 550 atomic mass unit (AMU) at 2.76 s/scan rate and were identified by comparing measured mass spectral data with those in NIST 14 Mass Spectral Library and with the reported data.

2.3 Antimicrobial Assay

2.3.1 Determination of antimicrobial activity

This was carried out using agar diffusion method [15]. A 0.1 mL of a 1:100 dilution of an overnight culture of each bacterial and fungal isolate was used to seed the surfaces of sterile well-set Mueller Hinton and Sabouraud Dextrose agar media respectively. The medium for *S. mutans* was supplemented with 5% sheep blood. The plates were allowed to dry in the incubator at 37 °C for 20 mins. A standard cork borer of 8 mm diameter was used to cut equidistance wells on the surfaces of the agar. One gramme of the EOs was reconstituted with 50% DMSO to final concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.125 mg/mL. Gentamycin at 7.8 µg/mL served as the positive control while methanol (50%) was used as the negative control. The agar plates were incubated at 37 °C for 24 hr after which diameter of zones of inhibition was measured. Result was average of triplicate experiments.

2.3.2 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) of the oil samples against the selected microorganisms were determined by the agar dilution method as described by [21] with modifications. To exactly 19 mL of a sterile molten Mueller Hinton agar maintained at 45 °C was added a 1mL of the EOs already diluted to give the following concentrations 200 mgmL⁻¹, 100 mgmL⁻¹, 50 mgmL⁻¹, 25 mgmL⁻¹, 12.5 mgmL⁻¹, 6.25 mgmL⁻¹ and 3.125 mgmL⁻¹ using the reported method by [16].

2.3.3 Determination of Minimum Bactericidal/Fungicidal concentration (MBC/MFC)

Minimum bactericidal concentrations (MBC) of the plant EOs were determined by the modified method of [17]. Exactly 0.5mL of oil samples at different concentrations as used in the MIC assay that showed no visible growth on the agar plates was added to 0.5 mL suspension of each of the test organisms in micro titre plate wells. These organisms were incubated at 37 °C for 24 to 48 hr. Samples were streaked out from the wells on to Mueller Hinton/ blood agar and Sabouraud dextrose agar to determine the minimum bactericidal concentration of the EOs required to kill the bacterial and fungal isolates respectively using the reported method by [15].

2.4 Docking studies

2.4.1 Preparation of Ligand for Docking

Compounds from the essential oil was drawn using a 2-D sketcher in the Schrodinger suite and imported into the Maestro suite workspace as 3-D. All the conformers generated were used for the docking process. The reference drug, Fluoroquinolone was obtained from pubchem.ncbi.nih.gov as SDF format. The Ligprep wizard of the Schrodinger suite was then used to prepare all the ligands by assigning bond length, bond angle, generating possible ionization state at pH 7 using Epik and finally optimized using OPLS4 force field [18–19].

2.4.2 Protein Preparation

The protein structures of *Escherichia coli* DPS with PDB 6GCM, with resolution 1.59 Å which provides protection for the cells during exposure to severe environmental assault (including oxidative stress and nutritional deprivation), was downloaded from a Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB). The retrieved crystal coordinates were prepared in the protein preparation wizard of the Schrodinger suite. With default parameters of assigning bond order, optimizing, and minimizing using OPLS4 force field. The sitemaps were generated using the sitemap wizard. This gives the site volume, the site score, size, Dscore, philic and phobic values of the sitemap. This generates possible pockets for binding in the protein molecules. The Receptor Grid Generation was carried out on each sitemap to know the residual numbers that are involved in the binding process. This was actualized at 1 Å with a partial charge cut off 0.25 Å [18–19].

2.4.3 Molecular Docking

The docking studies of the compounds from the EO and the reference drug with DPS (6GCM) were carried out in Maestro (version 13.2) released 2023-1. The binding strength of the docked complexes were analyzed through docking score, glide e-model and ligand efficiency. These energies measure the degree of stability of the binding between the protein molecules and the ligand molecules. Also, an interaction with amino acid residue were taken note of via hydrogen bonding interaction, pi-pi stacking, metal coordination, salt bridge and solvent exposure. Docking calculation were executed in the Extra Precision (XP) mode of the Glide module in the Schrodinger suite molecular docking platform. The complex with the highest negative docking scores has better binding towards the respective proteins. Docking calculation of compounds and conformer from leaf and seed EO

against the DPS (with PDB 6GCM) isoforms will provide a selectivity profile that may be interesting for the development of a novel anti-*E. coli* agent with a limited side effect. The 6GCM is an excellent target for the design of novel therapeutic strategies [18–20].

2.5 Statistical analysis

The experiments were repeated at least three times. The result values were expressed as mean $\pm$ SD. The comparisons between means were determined using One-way Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD) test, with Microsoft Excel. The data were considered significantly different statistically, if $p < 0.05$.

3. RESULTS AND DISCUSSION

The percentage yield of EOs from the two samples were calculated by the equation:

$$\text{Percentage yield of oil} = \frac{\text{volume of oil sample}}{\text{weight of dried plant sample}} \times 100$$

The percentage yield of the seed and leaf oils were found to be 1.04 and 1.22% (v/w) respectively.

3.1 Chemical Constituents of the *C. cainito* leaf Essential oils

3.1.1 Chemical composition

A total of thirty-one (31) compounds were identified in leaf representing 87.47% of EOs constituent; containing β -ocimene (12.37%) as highest component followed by geranylinalool (9.73%), alpha-farnesene (7.99%), (E)-Hex-3-enyl butyrate (7.20%), cyclofenchene (7.16%) and perhydroqualene (6.53%). All other components were detected at quantities below 6.0%. The chemical classes of the leaf oil were sesquiterpene hydrocarbon (27.36%), oxygenated monoterpene (23.18%), monoterpene hydrocarbons (21.22%) and oxygenated sesquiterpene (4.43%) as presented in Table 1.0. However, twenty-eight (28) constituents were detected in EO of the seed containing majorly oxygenated monoterpene (89.67%); Citral (36.60%),

Geranial, (28.50%), isocitral (10.82%), exo-isocitral (3.13%), and Nerol; (2.68%). The remaining classes are as follows: monoterpene hydrocarbons (3.52%), oxygenated sesquiterpene (1.04%) and sesquiterpene hydrocarbons (0.23%). The dominant class of compounds in the EOs of the leaf is sesquiterpene hydrocarbon (27.36%) while in the seed EOs, it is oxygenated monoterpene (89.67%) as reported in Table 1.0.

Some major differences were noticed in the quantitative and qualitative composition of the two essential oils but it was concluded from the data that both of them were rich in monoterpene. In EOs of the seed, the main class was formed by oxygenated monoterpene: Citral, Geranial, isocitral, exo-isocitral and Nerol. However, none of these oxygenated monoterpenes was detected in essential oil of the leaf. Both leaf and seeds EOs contained beta and alpha ocimene, cyclofenchene and pelargonaldehyde.

Table 1.0
Chemical Constituents of *Chrysophyllum cainitos* leaf essential oil.

S/N	Retention time (mins)	Compound name*	Percentage area (%)	Formula
1.	3.728	Caproaldehyde	2.10	C ₆ H ₁₂ O
2.	5.044	1-Hexanol	2.15	C ₆ H ₁₂ O
3.	5.353	Methylcyclopentane	1.01	C ₆ H ₁₂
4.	8.615	3,7-Dimethylocta-1,3,7-triene	1.06	C ₁₀ H ₁₆
5.	8.798	β-ocimene	12.37	C ₁₀ H ₁₆
6.	9.696	Cyclofenchene	7.16	C ₁₀ H ₁₆
7.	9.742	Pelargonaldehyde	2.65	C ₉ H ₁₈ O
8.	9.931	Methacrylic acid	1.72	C ₉ H ₁₄ O ₄
9.	10.137	Alloocimene	0.63	C ₁₀ H ₁₆
10.	11.030	(E)-Hex-3-enyl butyrate	7.20	C ₁₀ H ₁₈ O ₂
11.	11.110	Isobutyric acid	1.65	C ₄ H ₈ O ₂
12.	11.155	trans-2-Hexenyl butyrate	2.11	C ₁₀ H ₁₈ O ₂
13.	11.705	Pentanoic acid,(3Z)-3-hexen-1-yl ester	1.33	C ₁₁ H ₂₀ O
14.	12.775	undecyl aldehyde	1.35	C ₁₁ H ₂₂ O
15.	13.770	cis-3-Hexenyl hexanoate	1.73	C ₁₂ H ₂₂ O ₂
16.	13.868	1-[(1R,2S)-2,6,6-trimethyl-3-cyclohexane	0.76	C ₁₃ H ₂₀ O
17.	14.137	Lauraldehyde	1.96	C ₁₂ H ₂₄ O
18.	14.365	β-Caryophyllene	0.70	C ₁₆ H ₂₄
19.	14.886	7-methoxy-2,2-dimethylchromene	3.68	C ₁₂ H ₁₄ O ₂
20.	15.172	(3E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	1.10	C ₁₃ H ₂₀ O
21.	15.395	Alpha-farnesene	9.19	C ₁₅ H ₂₄
22.	15.487	2,4-di-tert-butylphenol	1.98	C ₁₄ H ₂₂ O
23.	16.242	Geranyllinalool	9.73	C ₁₆ H ₂₆
24.	17.066	Cedrelanol	0.65	C ₁₅ H ₂₆ O
25.	17.450	α-bulnesene	0.65	C ₁₅ H ₂₄
26.	19.143	Hexahydrofarnesyl acetone	0.73	C ₁₈ H ₃₆ O
27.	19.727	methyl ester 7-hexadecenoic acid	0.69	C ₁₇ H ₃₂ O ₂
28.	19.950	methyl palmitate	1.23	C ₁₇ H ₃₄ O ₂
29.	21.472	Oleic acid	1.13	C ₁₈ H ₃₄ O
30.	24.968	n-Octadecane	0.56	C ₁₈ H ₃₈
31.	27.744	Perhydrosqualene	6.53	C ₃₀ H ₅₀
		Monoterpene hydrocarbons	21.22	
		Oxygenated monoterpenes	23.18	
		Sesquiterpene hydrocarbons	27.37	
		Oxygenated sesquiterpenes	4.43	

S/N	Retention time (mins)	Compound name*	Percentage area (%)	Formula
		Non-terpene derivatives	11.28	
		TOTAL	87.47	

The dominant antimicrobial constituents of *Chrysophyllum cainito* leaf Eos is shown in Fig. 1.0, shows. These constituents may be responsible in part or in synergy for the observed antimicrobial activities showed by the oil sample.

3.1.2 Chemical Constituents of the *C. cainito* seed essential oils

The seed EOs of *C. cainito* was found to contain twenty-eight (28) oil components (Table 1.0 and Table 1.1). The seed EOs of *C. cainito* consist majorly of Citral (36.60%), Geranial, (28.50%), (E)-3,7-Dimethylocta-3,6-dienal (10.82%), exo-isocitral (3.13%), and nerol; (2.68%). The remaining constituents are as follows: oxygenated monoterpene (89.67%), non-terpene derivatives (4.43%), monoterpene hydrocarbons (3.52%), oxygenated sesquiterpene (1.04%), and sesquiterpene hydrocarbons (0.23%).

The dominant class of compounds in the leaf EOs of *C. cainito* is sesquiterpene hydrocarbon (27.36%) while in the seed EO, it is oxygenated monoterpene (89.67%). Research have shown that seasonal variations and geographical locations are the major factors that affect the composition of essential oil in samples [23]. Both leaf and seed EOs contained beta and alpha ocimene, cyclofenchene, pelargonaldehyde. β -Caryophyllene; a minor constituent was found in both EOs. (Table 1.0 and Table 1.1). Pino *et al.* [22] reported the characterisation of one-hundred and four compounds in the aroma concentrates of *Chrysophyllum cainito*. (E)-2-hexenal, 1-hexanol, limonene, linalool, copaene and hexadecanoic acid were found to be the major constituents. Similarly, geranylinalool and 1-hexanol, were also identified as minor and dominant constituents in the leaf EOs.

Table 1.1
Chemical Constituents of *Chrysophyllum cainitos* seed Essential oil.

S/N	Retention time (mins)	Compound name*	Percentage area (%)	Formula
1.	5.347	alpha-Ocimene	0.26	C ₁₀ H ₁₆
2.	5.800	β-Terpinene	0.24	C ₁₀ H ₁₆
3.	7.596	Sabinene	0.76	C ₁₀ H ₁₆
4.	7.739	Rosefuran	0.20	C ₁₀ H ₁₄ O
5.	7.848	Cyclofenchene	1.51	C ₁₀ H ₁₆
6.	7.997	Pelargonaldehyde	1.68	C ₉ H ₁₈ O
7.	8.941	Dipropylacetylene	0.28	C ₉ H ₁₆
8.	9.193	Exo-isocitral	3.13	C ₁₀ H ₁₆ O
9.	9.410	Terpineol	0.32	C ₁₀ H ₁₈ O
10.	9.708	3,6-Octadienal	8.42	C ₁₀ H ₁₆ O
11.	9.959	Rose furan oxide	0.66	C ₁₀ H ₁₄ O ₂
12.	10.131	(E)-3,7-Dimethylocta-3,6-dienal	10.82	C ₁₀ H ₁₆ O
13.	10.228	Propanoic acid	0.25	C ₁₀ H ₁₈ O ₂
14.	11.095	Rhodinal	0.47	C ₁₀ H ₂₀ O
15.	11.344	Geranial	28.56	C ₁₀ H ₁₆ O
16.	11.590	Nerol	2.68	C ₁₀ H ₁₈ O
17.	11.899	Citral	34.63	C ₁₀ H ₁₆ O
18.	13.627	3-Carene	1.32	C ₁₀ H ₁₆
19.	14.182	3,4-epoxycyclopentanone	0.35	C ₅ H ₆ O ₂
20.	15.424	Phenol, 2,4-bis(1,1-dimethylethyl)	0.19	C ₁₄ H ₂₂ O
21.	16.351	β-Caryophyllene	0.19	C ₁₃ H ₂₂
22.	16.711	Xylene-2-carboxaldehyde	0.19	C ₉ H ₁₀ O
23.	16.780	Viridiflorol	0.23	C ₁₅ H ₂₄
24.	17.209	Rosefuran	1.17	C ₉ H ₁₀ O
25.	17.770	Palmitaldehyde	0.19	C ₁₆ H ₃₂ O
26..	19.956	methyl palmitate	0.30	C ₁₇ H ₃₄ O ₂
27.	20.620	Ethyl palmitate	0.30	C ₁₈ H ₃₆ O ₂
28	21.793	6-Methyl-4,6-bis(4-methylpent-3-en-1-yl)cyclohexa-1,3-dienecarbaldehyde	0.44	C ₁₈ H ₃₆ O ₂
		Monoterpene hydrocarbons	3.52	
		Oxygenated monoterpenes	89.67	
		Sesquiterpene hydrocarbons	0.23	
		Oxygenated sesquiterpenes	1.04	
		Non-terpene derivatives	4.43	
		TOTAL	98.89	

The dominant antimicrobial constituents of *Chrysophyllum cainito* seed Eos is shown in Fig. 2.0. These constituents may be responsible in part or in synergy for the observed antimicrobial activities shown by the oil sample.

3.2 Biological Significance of the Dominant Constituents of *C. cainito* leaf and seed oils

Citral has gained much attention from researchers due to which plenty of its bioactivities such as, antimicrobial activity [24–25], antiviral activity [26], anti-inflammatory activity [27–28], anti-leishmanial activity [29–30], inhibitory activity against cytokines effect [31], chemopreventive activity [32], allelopathic activity [33], aldose reductase inhibitor activity [34], spasmolytic activity [35], antiadipogenic activity [36], insect repellent effect [36], anti-parasitic activity [37], Insecticidal effects [38].

Geranial; an oxygenated monoterpene has been reported for its antimicrobial activity [39], antifungal activity [40], as a plant insect repellent [41], anti-inflammatory agent [42] and anthelmintic property [43]. Also, **Nerol**; a major constituent of *C. cainito* have been reported previously for its antimicrobial activity [44] and anticancer properties [44]. **β-ocimene** in EOs is associated with anticonvulsant activity, antifungal activity, antitumor activity, and pest resistance [45–47]. Ocimene is also a volatile pheromone important for the social regulation of honeybee colonies [48]. **Alpha farnesene** has been reported for its insecticidal activity [49].

3.3 Antimicrobial Analyses of seed and leaf essential oils of *Chrysophyllum cainito*.

Previously, the pulp and seed samples of *Chrysophyllum cainito* was analyzed for its antimicrobial potentials against some clinical isolates of bacteria and fungi, the microbial counts of the fruits ranged from 1.0×10^9 cfu/mL to 2.4×10^{10} cfu/mL for total aerobic plate counts, 1.0×10^7 to 2.0×10^7 (cfu/mL) for fungal counts, and 1.0×10^8 to 1.2×10^9 (cfu/mL) for coliform counts, the current work justifies this finding [13].

Currently, the EOs from the seed and leaf of *Chrysophyllum cainito* were screened against 7 bacterial and 2 fungal clinical isolates. The results of the antimicrobial susceptibility test are represented in Table 1.3, Fig. 1.4, and Fig. 1.5. The MICs of the seed EOs against all tested micro-organisms ranged between 12.5 and 50 mg/mL, whereas those of the leaf EOs were between 0.781251 and 50 mg/mL. In general, the leaf EOs exhibited better antimicrobial activity when compared to the seed EOs. MICs of the leaf oil were lower than those of the seed oil. The lesser the MICs the better the activity of the essential oils. *Salmonella typhi*, *Candida albicans*, *Tricophyllum rubrum*, *Pseudomonas aeruginosa* and *Bacillus subtilis* all displayed very low sensitivity (resistance) to the antimicrobial activity of the leaf and seed Eos as compared to other test organisms. *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* showed high level of susceptibility to the activity of the EOs. The Minimal Bactericidal Concentration is the least concentration of the oil sample capable of causing death of the microbes. Essentially, the leaf and seed oils were mostly potent against *Staphylococcus aureus* (MBC: 12.5 mg/mL each for leaf and seed) and *E. coli* (MBC: 12.5mg/mL for leaf and 25 mg/mL for seed), has the best MBC potential of 12.5 µg/mL for the seeds and leaf oils respectively. The seeds and leaf oils also displayed a moderate mortality concentration against *Escherichia coli*, while the remaining organisms recorded low MBC contractions of 50 to > 50 µ g/mL for the oil samples. Both EOs displayed strong and broad-spectrum antimicrobial activity against *Candida albicans* and *Tricophyllum rubrum* which were the fungi used in this study (Table 1.3, Fig. 1.4, Fig. 1.5).

Table 1.3
Antimicrobial Activity of *Chrysophyllum canito* leaf and Seed Essential oils.

S/N	ORGANISM	C.ASOIL		C.ALOLI		BROTH ONLY	BROTH and OIL	BROTH and ISOLATES	GENTAMICIN		KETOCONAZOLE	
		MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)				MIC (µ g/ml)	MBC (µ g/ml)	MIC (mg/mL)	MFC (mg/mL)
1	S.a	12.5	12.5	0.781251	12.5			+	5	5	NA	NA
2	B.s	> 50	> 50	50	50			+	5	10	NA	NA
3	E.c	12.5	25	1.625	12.5			+	10	10	NA	NA
4	P.a	50	> 50	50	> 50			+	> 10	> 10	NA	NA
5	K.p	12.5	50	3.125	25			+	10	10	NA	NA
6	S.typ	50	> 50	50	50			+	> 10	> 10	NA	NA
7	C.a	> 50	> 50	> 50	> 50			+	NA	NA	1	1
8	T.r	50	50	> 50	> 50			+	NA	NA	0.25	0.5

Key:

S.a = *Staphylococcus aureus*, *B.s* = *Bacillus subtilis*, *E.c* = *Escherichia coli*, *S.typ* = *Salmonella typhi*, *PS.a* = *Pseudomonas aeruginosa*, *K.p* = *Klebsiella pneumoniae*, *C.a* = *Candida albicans* and *T.r* = *Tricophytum rubrum*.

3.4 Molecular Docking Studies

The docking score of compound 11 from seed EO with the docking score (-3.303) showed the docking score of all compound from the both leaf and seed) of all the sitemaps and close to that of reference compound on sitemap three with docking score of -5.055. The other compounds with close docking score include; -3.175 (1), -2.781 (2), -2.726 (12), -2.620 (10), -2.443 and - 2.440 (3). The anti *E. coli* activities of both leaf and seed EO could be associated with inhibitory activities of the compound on the active sites of the protein.

Compound 1, 2, 3, 4 and 7 has no interaction with any of the five sitemaps. Compound 5 interacted only with sitemap 3 through hydrogen bond with ASN99; 2.16. Compound 6 interacted with sitemaps 3 and 5 through hydrogen bonding with LYS101; 2.67, THR16; 1.76, and LYS157; 2.14. Compound 8 interacted with sitemaps 3 and 5 through hydrogen bonding with TYR162; 2.01 and LYS157; 1.83

Compound 9 interacted only with sitemap 3 through hydrogen bonding with LYS101; 2.13 and TYR16; 2.00. Compound 10 interacted with sitemaps 2, 3 and 5 through hydrogen bonding with ASP146; 2.10, TYR16; 1.98 and LYS157; 1.90

Compound 11 interacted with sitemaps 1, 3, 4 and 5 through hydrogen bonding with ASP99; 2.29, SER100; 1.79, GLU163; 1.76, ASP146; 1.56 and GLU64; 1.76.

Compound 12 interacted with sitemaps 3 and 5 through hydrogen bonding with ASN96; 1.75 and LYS157; 1.74.

The reference drug (13 and 14) with the five sitemaps through hydrogen bonding and pi-pi stacking. The hydrogen bonded amino acids include; THR149; 2.06, THR149; 1.93, ASP142; 1.78, GLU64; 1.72, GLU64; 1.68, TYR16; 2.31, TYR16; 1.85, GLU163; 1.94 and SER100; 1.96. The pi-pi stacking amino acids include; PHE161; 5.36 and PHE69; 5.39.

Compounds 1, 2, 3, 4, 5, 6 and 7 are compound present in leaf EO and one conformer for cyclofenchene, compounds 8, 9, 10, 11, and 12 are compounds present in seed EO and compounds 13 and 14 (Flouroquinolone) are known anti *E. coli* drug and a conformer used as reference compound (Fig. 1.5–1.7 and Table 1.4).

Table 1.4
Binding Energies of the Compound with the Five Sitemaps from 6GCM

Compounds	Binding Affinity (ΔG) Kcal/mol				
	Sitemap 1	Sitemap 2	Sitemap 3	Sitemap 4	Sitemap 5
1	-3.175	1.171	-0.369	-1.523	-0.663
2	-2.048	-1.040	-2.781	-2.095	-1.309
3	-2.066	-1.421	-2.443	-2.440	-1.354
4	-1.919	-1.895	-0.444	-1.834	0.840
5	-1.881	-1.837	-2.146	-1.731	-0.835
6	-1.001	-1.433	-0.918	-0.886	0.828
7	0.676	0.494	1.032	-1.897	-0.801
8	-2.311	-2.205	-2.234	-1.801	-1.595
9	-2.187	-1.277	-1.811	-1.827	-0.630
10	-2.045	-1.972	-2.620	-1.335	-0.979
11	-1.531	-1.802	-3.303	-1.676	-0.596
12	-1.185	-1.016	-2.726	-1.030	-1.083
13	-1.933	-2.393	-5.055	-2.100	-2.322
14	-1.471	-0.045	-3.031	-1.338	-0.355

4. Conclusion

Based on the results obtained in the current report, *Chrysophyllum cainito* L. leaf EOs contains more oil constituents than the seed EOs. β -ocimene has highest percentage composition in the leaf oil while citral was most abundant in the seed oil. The leaf and seed EOs of *C. cainito* contained majorly sesquiterpene hydrocarbon (27.36%) while the seed EOs was dominated by oxygenated monoterpenes (89.67%). These compounds in part or

in synergy may be responsible for the antimicrobial activity of *C. cainito*. The leaf and seeds EOs contain Beta and alpha ocimene as well as cyclofenchene were also found as part of the chemical constituents of the EOs. The leaf EOs MIC and MBC results of the antimicrobial screening showed that the leaf oil had a better activity when compared to the seed EOs. Both EOs displayed strong and broad-spectrum antimicrobial activity towards *Staphylococcus aureus*, *E. coli*, *Candida albicans*, and *Tricophytum rubrum*. The antimicrobial constituents of *C. cainitos* showed good binding interactions.

Further studies are hereby advised on the antimicrobial activity of *C. cainito* using a different approach and methodology, the druggability and toxicity of the oil samples are required.

Table 1.8
Names of the Docked Dominant Constituents of *C. cainitos*
oils with antimicrobial standards

Compound number	Docked compound
1	1,3,7,11-trideca-tetraene-5,9-triyn
2	Hex-3-enylbutyrate
3	Alpha fernesene
4	Perhydrosqualene
5	Beta-Ocimene
6	Cyclofenchene
7	Citral
8	3,7-dimethyl-1,2-Octadienal
9	3,7-dimethyl-octa-3,6-dienal
10	3,6-Octadienal
11	2,7-dimethyl-2,6-octadiene-1-ol
12	7-methyl-3-methyldiene-6-octanal
13	beta-Fluoroquinoline
14	Fluoroquinoline

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All the authors in this work approved the submission of this research article

Competing interests

The authors declare no competing interest

Funding

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Author Contribution

O.B: Conceived the research ideas and directed the research, collection, identification of plant samples and in the writing of the manuscript.E.Y: Assisted in gathering literature data and in the extraction of the volatile oil samples in the laboratory.S .O: Edited the final manuscript.A.K: Assisted in gathering literature data and in the extraction of the volatile oil samples in the laboratory.J.O: Edited the final manuscript.I.M: Did the molecular Docking studies.

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Not applicable

Availability of data and material

Not applicable

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Tables

Figures

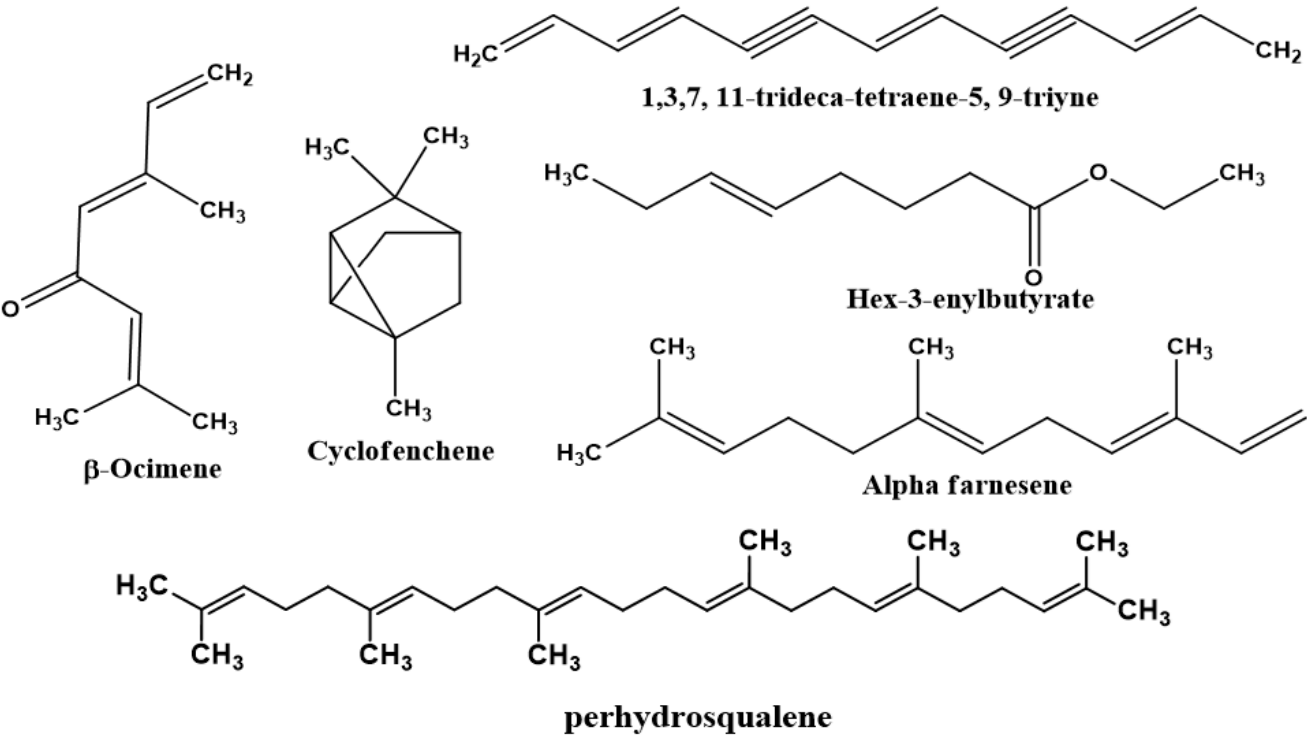


Figure 1

Figure 1.0: Some dominant antimicrobial constituents of *Chrysophyllum cainito* leaf Essential oil

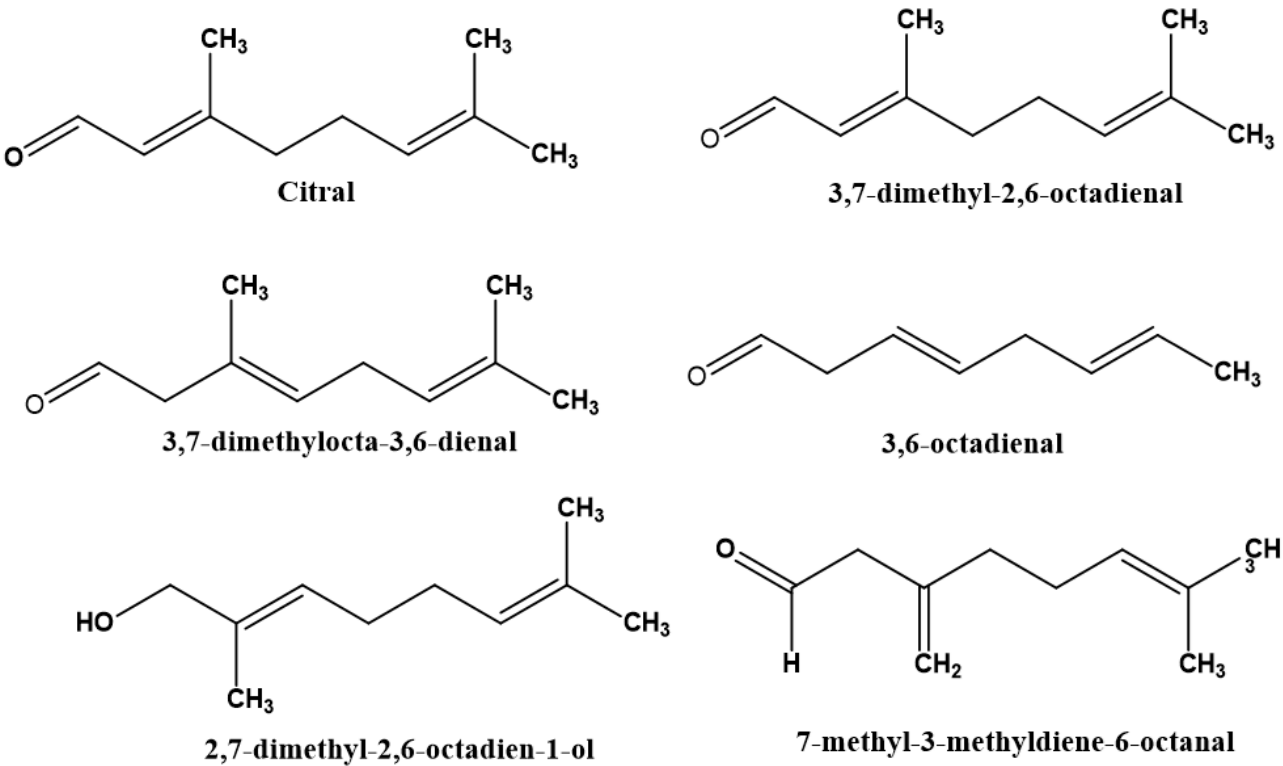


Figure 2

Figure 2.0: Some antimicrobial constituents of *Chrysophyllum cainitos* seed Essential oil

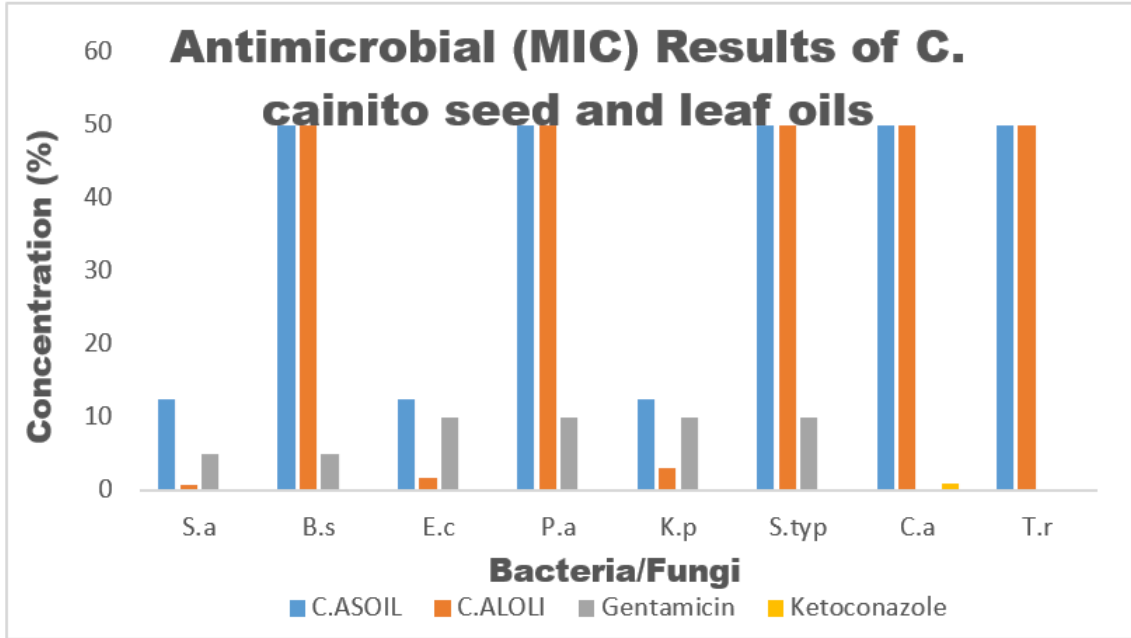


Figure 3

Figure 1.4: Antimicrobial (MIC) Results of *C. cainito* seed and leaf oils

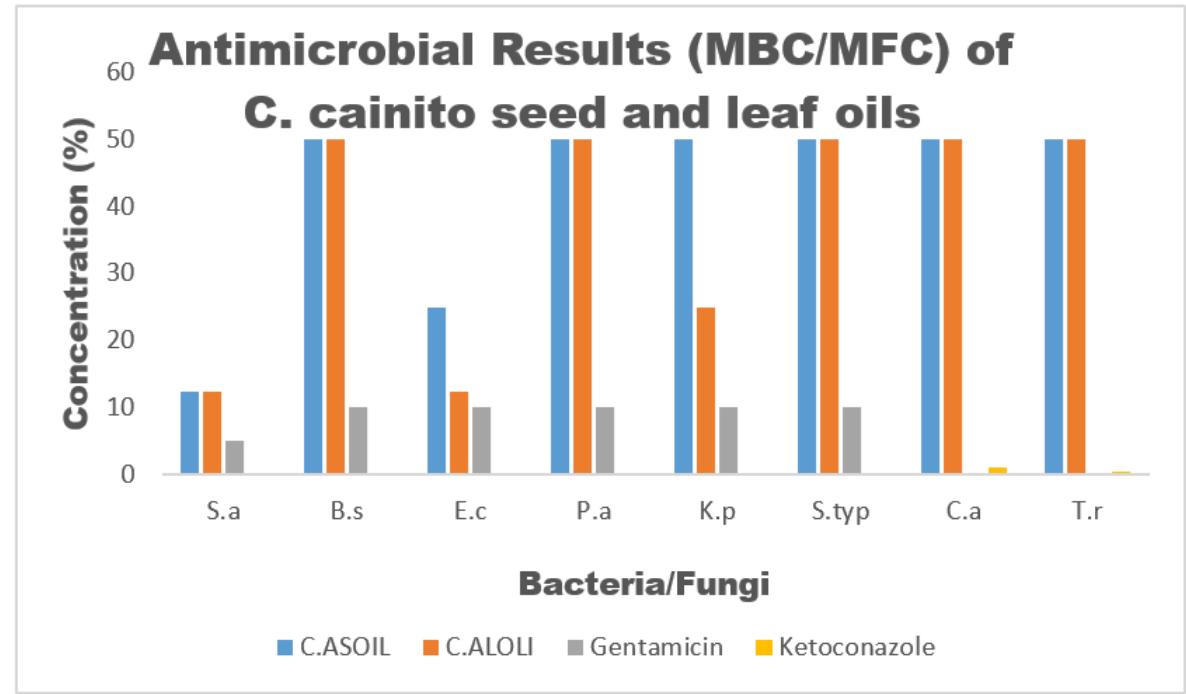


Figure 4

Figure 1.5: Antimicrobial (MBC/MFC) Results of *C. cainito* seed and leaf oils

Supplementary Files

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