STRUCTURAL AND ANTIBACTERIAL STUDIES OF THE CONSTITUENTS OF THE ESSENTIAL OIL OF *CLEISTOPHOLIS PATENS* ENGL. & DIELS COLLECTED IN GAGNOA (CÔTE D'IVOIRE)

Abstract:

The essential oil of *Cleistopholis patens* (Annonaceae) leaves was obtained by hydrodistillation using a traditional still from leaves collected in the Gagnoa department, Côte d'Ivoire, with a yield of 0.21%. Chemical analyses were carried out by gas chromatography-mass spectrometry (CPG-MS) and carbon-13 nuclear magnetic resonance (^{13}CNMR). Sixteen compounds, accounting for 95.75% of the total composition, were identified. The chemical profile is characterized by a balanced distribution between hydrocarbon monoterpenes (34.88%) and hydrocarbon sesquiterpenes (36.84%), complemented by oxygenated sesquiterpenes (21.18%) and an aromatic hydrocarbon, naphthalene (5.13%). The major constituents were *p*-cymene (14.36%), caryophyllene (10.77%), and 3-carene (9.23%). The essential oil of *Cleistopholis patens* from Gagnoa exhibited pronounced bactericidal activity, attributed to its chemical diversity, notably the presence of compounds well recognized for their effectiveness against a variety of microbial agents.

Keywords:*Cleistopholis patens*, essential oil, CPG-MS, NMR, bactericidal activity.

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Introduction:

Since antiquity, humankind has consistently turned to nature to meet its fundamental needs: food, shelter, clothing, and medical care. The therapeutic use of medicinal plants is ancient, embedded within a long-standing tradition that has accompanied human development.[1]Even today, this practice remains deeply rooted in many parts of the world, particularly in Africa, where access to modern medicines is often hindered by issues of quality, availability, and, above all, cost. According to the World Health Organization[2], more than 80% of the population in developing countries relies on traditional medicine and medicinal plants for primary healthcare.

Among the plants used in traditional medicine, aromatic species are especially valued for their essential oils (EOs). These natural volatile extracts, generally obtained by steam distillation from the whole plant or specific organs (flowers, leaves, bark, roots, etc.), are widely recognized for their biological properties, notably antimicrobial and antioxidant activities. As a result, EOs are extensively employed in the pharmaceutical, cosmetic, food, and aromatherapy industries.[3]

In Côte d'Ivoire, floristic diversity offers significant potential for the valorization of medicinal species. In the Gagnoa region, located in the Center-West of the country, several plant species of medicinal interest remain understudied. *Cleistopholis patens*, a tropical tree commonly found in secondary humid forests, is particularly abundant in this area. This species typically grows in disturbed and swampy environments.[4]

Studies conducted on *Cleistopholis patens* have revealed considerable variability in the chemical composition of its essential oils, depending on their geographical origin. In Côte d'Ivoire, the EOs of this plant differ from those reported in countries such as Nigeria and Cameroon.[5]Such variations are thought to result from local ecological conditions.[6]The

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antibacterial activities of C. patens EOs are mainly associated with the presence of terpenic and aromatic compounds, which are well established for their antimicrobial efficacy.[7]

However, to date, few studies have specifically investigated the essential oils of *Cleistopholis patens* collected in the Gagnoa department. Such an investigation appears necessary to better characterize their chemical composition and assess their biological potential, particularly in the context of combating bacterial infections.

Material and Methods

Material

Plant material

The plant material used in this study consisted of the leaves of *Cleistopholis patens*. They were collected between late July and early August 2024 in forested areas of the Gagnoa department, located in the Gôh-Djiboua District, Center-West Côte d'Ivoire. After collection, the leaves were shade-dried in an air-conditioned room maintained at a constant temperature of 27 °C for two weeks, in order to preserve their volatile constituents prior to essential oil extraction.

Technical equipment

The extraction and analysis of the essential oil were performed using several instruments: a Clevenger-type hydrodistillation apparatus, a heating plate, a Pioneer PA202C balance (capacity 2100 g, precision 0.01 g), a Delsi DI 200 gas chromatograph equipped with a flame ionization detector, a gas chromatography–mass spectrometry (CPG-MS) system, a nuclear magnetic resonance (NMR) spectrometer, and a HACH DR 2400 spectrophotometer, in addition to standard laboratory glassware.

Bacterial strains under study

Eight clinical bacterial strains of different phenotypes[8], obtained from the biobank of the Pasteur Institute of Côte d'Ivoire, were used in this study (Tab.1).

Bactéria	Code	Sample type	ResistancePhenotype

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Reference strain			
Staphylococcus aureus (*S.aureus*)	ATCC 25923	-	Wild type
Hospital-derivedstrains			
Staphylococcus aureus (*S.aureus*)	2247C/24	Blood	Wild type
*Staphylococcus*sp (*S*.sp)	2101PIS/24	Urine	MLSbinductible
Escherichia coli (*E.coli*)	2811C/24	Urine	FQR
Klebsiellapneumoniae(*K.pneu.)*	2701C/24	Urine	ESBL, FQR
*Yersinia*sp (*Yer*.sp)	2340PIS/24	Stool	Wild type
*Salmonella*sp (*Sal*.sp)	2077AD/24	Stool	Wild type
*Salmonella*sp (*Sal*.sp)	2520C/24	Pus	Wild type

Table 1. Bacterial strains tested and their resistance phenotypes

ESBL: Extended-Spectrum β-Lactamases; FQR: Fluoroquinolone Resistance; ATCC: American Type Culture Collection; MLS: Resistance to Macrolides, Lincosamides, and Streptogramin B

Methods

Extraction of essential oil by hydrodistillation

Approximately 1.5 L of water was introduced into the distillation flask. A measured quantity of dried leaves was weighed and placed into the still, which was sealed and brought to boiling. The extraction time was three hours, counted from the appearance of the first drop of essential oil (EO). The extracted EOs were weighed, stored in amber glass vials, and kept in a freezer at approximately 0 °C.

The yield for each EO sample was calculated according to the following equation:

Yield (%) = (Mass of EO obtained (g) / Mass of dried plant material (g)) ×100

Characterization by CPG-MS and ^{13}C NMR

The essential oil was characterized using proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR), gas chromatography (CPG), and gas chromatography-mass spectrometry (CPG-MS). NMR spectra were recorded on a Bruker spectrometer (Bruker BioSpin AG) equipped with a 5-10 mm probe, operating at 400.132 MHz for proton and 100.623 MHz for carbon-13. Chemical shifts (δ, in ppm) were referenced to tetramethylsilane(TMS) as the internal standard. ^{13}C NMR spectra were obtained under the following conditions: 5 mm probe, 45° pulse angle, acquisition time 2.73 s corresponding to a 64 K data set, spectral width (SW) 25,000 Hz (250 ppm), and digital resolution 0.183 Hz/pt. The number of accumulations ranged from 2000 to 5000 per spectrum. Decoupling was achieved using the pulsed-field “Composite Pulse Decoupling” technique.

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Antibacterial activity of the essential oil

Preparation of the bacterial inoculum

Bacterial strains were cultured on selective media at 37 °C for 24 h to obtain fresh, well-isolated colonies. One to two colonies with uniform morphology were suspended in 2 mL of physiological saline and homogenized using a vortex. The inoculum density was adjusted to 0.5 McFarland using a Densimat and used to inoculate Mueller–Hinton (MH) agar.[8]

Preparation of test concentrations

The essential oil was diluted in Tween 80 (90% oil, 10% Tween) to ensure miscibility with water. A stock suspension of 200 mg/mL was prepared, vortex-homogenized, and serially diluted twofold to obtain concentrations of 200 and 100 mg/mL. The Tween 80/distilled water mixture served as the negative control, while strain-specific antibiotics were used as positive control.[9]

Antibacterial assays

The antibacterial activity of the essential oil was assessed using two methods: agar well diffusion and broth microdilution. Agar diffusion allowed measurement of inhibition zones, with the extract considered active when the diameter was ≥ 9 mm.[10]The broth microdilution method was used to determine the minimum inhibitory concentration (MIC), defined as the lowest concentration preventing visible bacterial growth, and the minimum bactericidal concentration (MBC), defined as the concentration killing 99.99% of bacteria. Based on the MBC/MIC ratio, the extract was classified as bactericidal if ≤ 2, or bacteriostatic if > 2.[11]

Results and discussion

Yield and chemical composition

The extraction of the essential oil from *Cleistopholis patens* (Gagnoa) yielded 0.21%.

All identified compounds exhibited signals clearly assignable to protonated carbons in the ^{13}C NMR spectrum of the sample. Only the signals corresponding to the quaternary carbons of minor compounds were not observed, without affecting the overall identification of the molecules. Subsequently, the sample was analyzed by gas chromatography-mass spectrometry (CPG-MS) to precisely characterize its volatile constituents. The resulting chromatogram (Fig. 1) displayed several well-resolved peaks, each corresponding to a distinct compound, with varying retention times reflecting the diversity of compounds according to their volatility and polarity. The most intense peaks corresponded to the major constituents, while the weaker peaks represented minor components.

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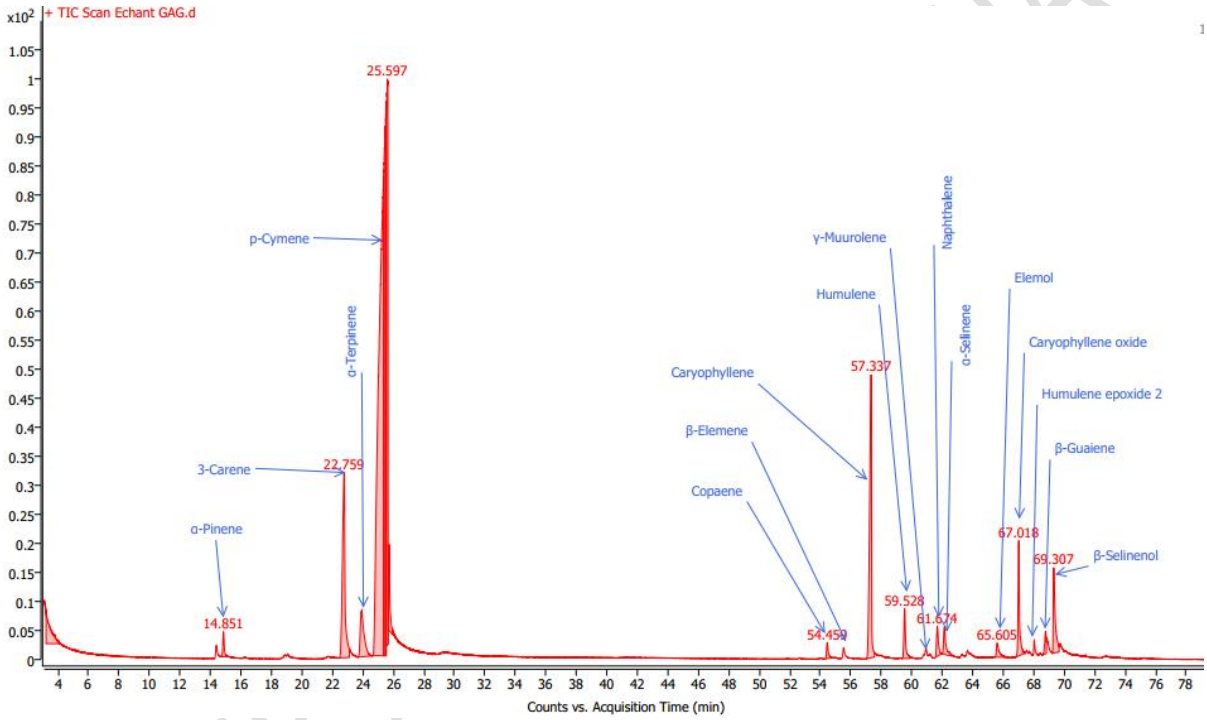

Figure 1: CPG-MS chromatographic profile of the essential oil

Analysis of the mass spectra associated with each peak allowed the identification of compounds based on their molecular ion and characteristic fragments. The major constituents belong to the chemical families of monoterpenes, sesquiterpenes, and a few aromatic compounds, with their relative proportions reflecting their abundance in the sample. Three compounds are particularly notable: the bicyclic sesquiterpenecaryophyllene (10.77%), and 2 monoterpenes, p-cymene (14.36%) and 3-carene (9.23%).

Compound assignments in the mass spectra were initially made by comparing retention indices with library data, followed by confirmation through consistency between the proposed compound's possible fragmentation and the observed spectral peaks. Below are the possible

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fragmentation patterns for caryophyllene, p-cymene, and 3-carene, three of the predominant compounds in the essential oil.

- **Caryophyllene**

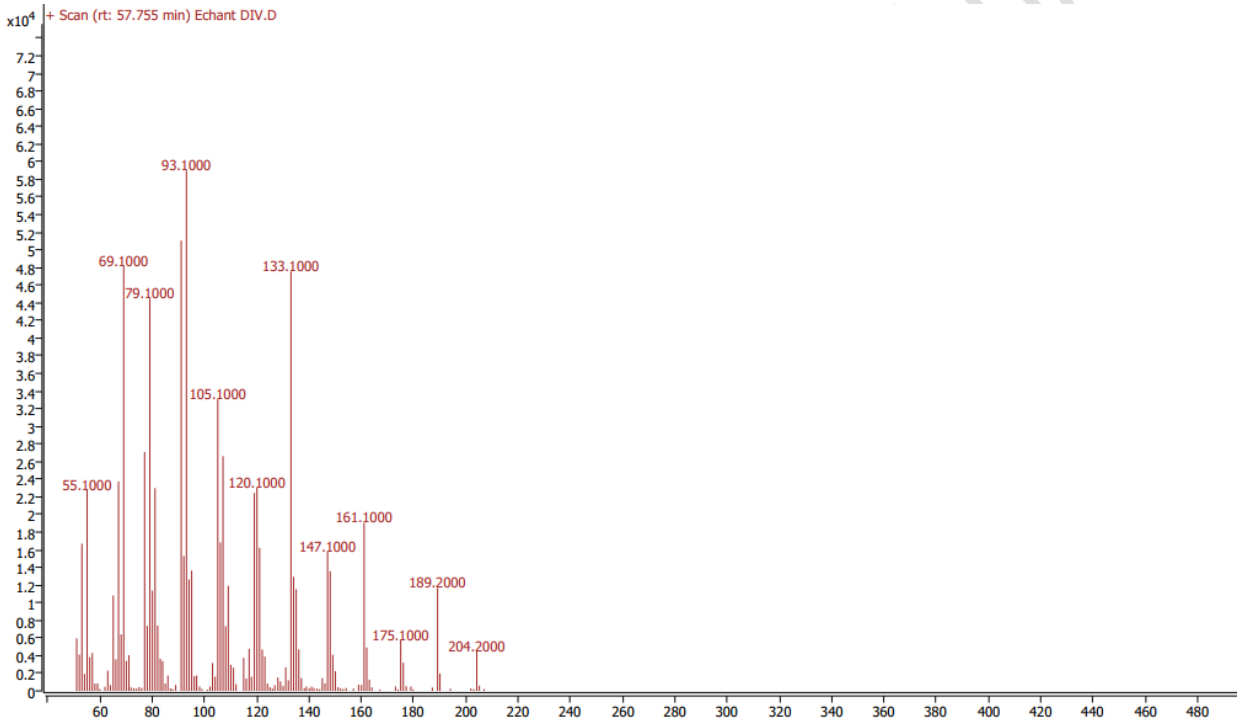

Figure 2: Mass spectrum of β-Caryophyllene

- The mass peak at 204 corresponds to the molecular ion $C_{15}H_{24}^{+}$.

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- The base peak (m/z = 133.1) corresponds to $[M - 71]^+$, resulting from the loss of CH_3^+ and $C_4H_8^+$ ions.

- The mass peaks at 91 and 93, corresponding to $[M - 71 - 42]^+$ and $[M - 71 - 40]^+$, are attributed to the loss of $C_3H_6^+$ and $C_3H_4^+$ fragments, associated with ring opening.

- The mass peak at 41, corresponding to $[M - 71 - 40 - 52]^+$, indicates the probable loss of the $C_4H_5^+$ fragment

- **p-Cymene,**

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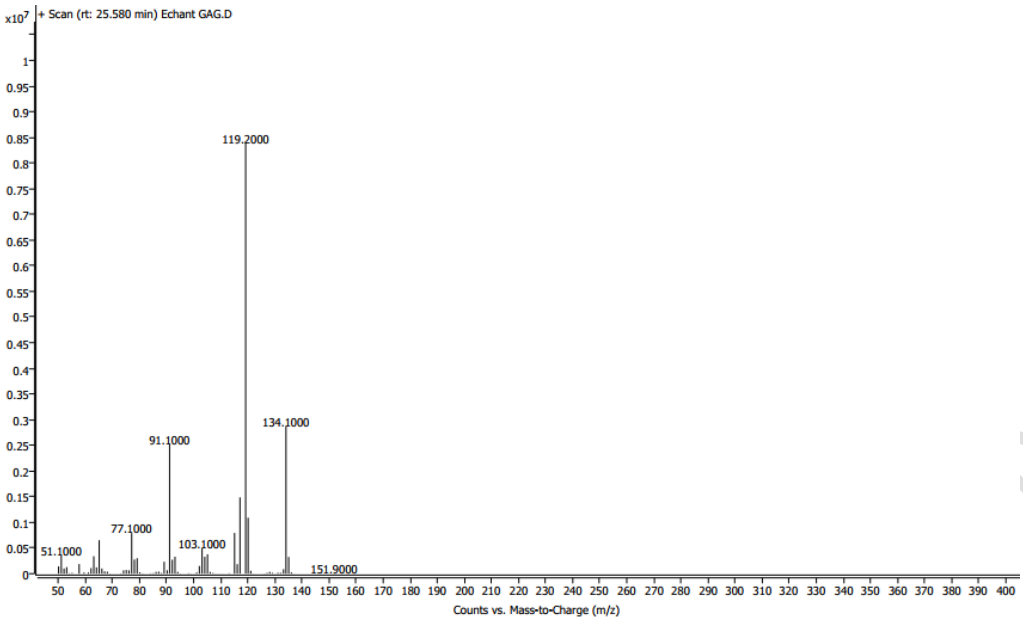

Figure 3:Mass spectrum of p-Cymene

- The mass peak at 134 corresponds to the molecular ion $C_{10}H_{14}^{+}$.

- The base peak (m/z = 119.2) corresponds to [M -15] $^{+}$, resulting from the loss of a CH_3^{+} ion.

- The base peak (m/z = 91.1) corresponds to [M - 43], resulting from the loss of a $C_3H_7\cdot$ radical (α-cleavage between the ring carbon and the isopropyl substituent), initially forming a benzyl cation (benzylium), which readily rearranges into a tropylium ion (a stable aromatic ion).

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- **Carene**

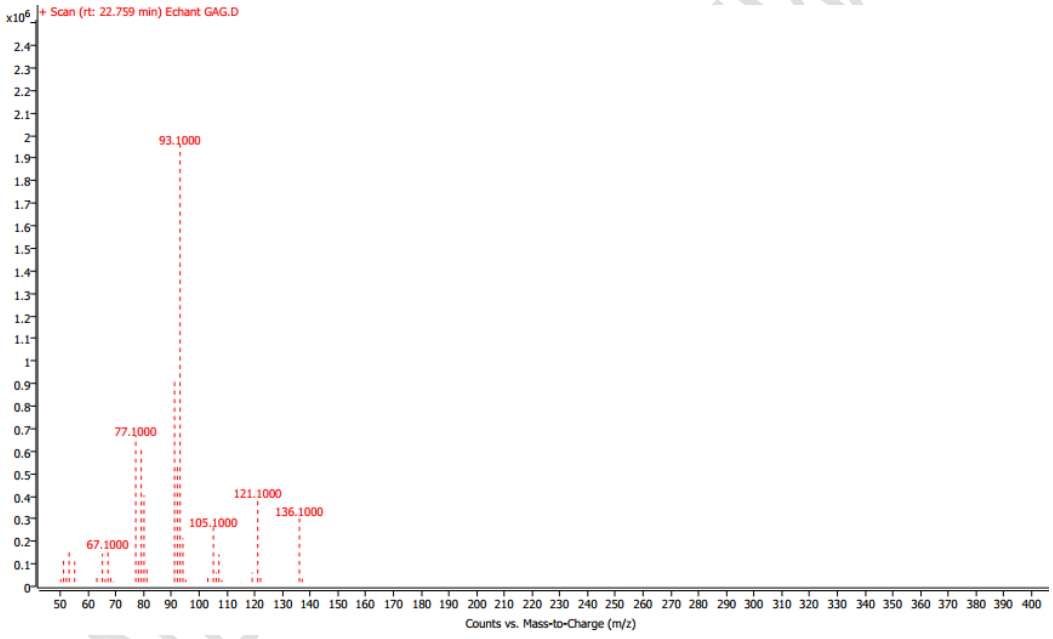

Figure 4 :Mass spectrum of 3-Carene

- The mass peak at 136 corresponds to the molecular ion $C_{10}H_{16}^{+}$.

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- The base peak (m/z = 121.1) corresponds to$[M - 15]^+$, resulting from the loss of a CH_3^+ ion.

- Another peak (m/z = 121.1) corresponds to $[M - 15-28]^+$, resulting from the loss of a $C_2H_4^+$ ion.

In summary, the different molecular fragmentations identified by GC–MS, along with their ^{13}C NMR analyses, are presented in Table 2.

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Table 2: Chemical composition of the essential oil of *Cleistopholis patens*

N°	Identified compounds	Retention time (min)	Method of characterization	Percentage %
1	α-Pinene	14.851	CPG-MS, ^{13}C NMR	4.62
2	3-Carene	22.759	CPG-MS, ^{13}C NMR	9.23
3	α-Terpinene	24.117	CPG-MS, ^{13}C NMR	6.67
4	p-Cymene	25.597	CPG-MS, ^{13}C NMR	14.36
5	Copaene	54.459	CPG-MS, ^{13}C NMR	4.1
6	β-Elémene	55, 121	CPG-MS, ^{13}C NMR	2.88
7	Caryophyllene	57.337	CPG-MS, ^{13}C NMR	10.77
8	Humulene	59.528	CPG-MS, ^{13}C NMR	6.15
9	γ-Muurolene	61.674	CPG-MS, ^{13}C NMR	2.28
10	Naphthalene	61.986	CPG-MS, ^{13}C NMR	5.13
11	α-Selinene	62.112	CPG-MS, ^{13}C NMR	5.64
12	Elemol	65.605	CPG-MS, ^{13}C NMR	3.54
13	Caryophylleneoxide	67.018	CPG-MS, ^{13}C NMR	7.69
14	Humuleneepoxide 2	68.010	CPG-MS, ^{13}C NMR	3.28
15	β-Guaiene	60.013	CPG-MS, ^{13}C NMR	5.02
16	β-Selinenol	69.307	CPG-MS, ^{13}C NMR	6.67
	Total identified compounds			95.75
	Aromatichydrocarbons			5.13
	Hydrocarbonmonoterpenes			34.88
	Hydrocarbonsesquiterpenes			36.84
	Oxygenatedsesquiterpenes			21.18

Chromatographic (CPG-MS) and spectroscopic (^{13}C NMR) analyses of the essential oil of *Cleistopholis patens* allowed the identification of sixteen constituents, representing 95.75% of the total composition. The chemical profile is characterized by a balanced predominance of hydrocarbon monoterpenes(34.88%) and hydrocarbon sesquiterpenes (36.84%), complemented by a notable proportion of oxygenated sesquiterpenes (21.18%) and a non-terpenic aromatic compound, naphthalene (5.13%). This distribution reflects considerable chemical diversity and suggests a wide range of potential biological activities.Among the hydrocarbon monoterpenes, the major constituents are p-cymene (14.36%), 3-carene (9.23%), α-terpinene (6.67%), and α-pinene (4.62%). These molecules are frequently reported for their antimicrobial, antioxidant, and anti-inflammatory properties, supporting their central role in the biological activity of essential oils.[12,13]The hydrocarbon sesquiterpene fraction is dominated by caryophyllene (10.77%), followed by humulene (6.15%), β-guaiene (5.02%), α-selinene (5.64%), as well as minor compounds such as β-elemene (2.88%) and γ-muurolene (2.28%). These sesquiterpenes are associated with anti-inflammatory, antifungal, and cytotoxic activities [14], highlighting the pharmacological relevance of the oil.Oxygenated sesquiterpenes account for 21.18% of the sample, led by caryophyllene oxide (7.69%),

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followed by β-selinenol(6.67%), elemol (3.54%), and humulene epoxide 2 (3.28%). These compounds are particularly studied for their antifungal, neuroprotective, and anticancer effects.[15,16] Their relatively high proportion suggests an enhanced biological potential of the extract. A distinctive feature of the profile is the significant presence of naphthalene (5.13%), an aromatic hydrocarbon rarely reported in essential oils. Although often considered an atypical marker, its detection warrants attention and may reflect a specific biochemical trait of C. patens. Compared to other essential oils from Myrtaceae, which are typically dominated by sesquiterpenes or volatile monoterpenes such as limonene, linalool, or 1,8-cineole[17], the profile of C. patens is characterized by a high representation of hydrocarbon sesquiterpenes, particularly δ-cadinene (~28.7%), α-copaene (~16.9%), and β-caryophyllene (~27.5% in leaves), along with germacrene B and D as other major components.[18]This chemical architecture shows similarities with certain Lamiaceae, notably Hyptissuaveolens, whose essential oil is rich in β-caryophyllene (~32-34%), α-humulene, and germacrene D/B, compounds with well-documented antimicrobial and pharmacological properties.[19,20]Thus, the chemical diversity and complementarity observed in C. patens essential oil suggest a synergy between monoterpenes and sesquiterpenes, both hydrocarbon and oxygenated, rather than activity relying on a single major compound. This complexity likely explains its traditional uses in local pharmacopoeia and opens perspectives for its exploitation in pharmaceutical, cosmetic, and food industries. The synergistic effect among constituents, frequently reported in the literature, represents a key advantage for its valorization.[21]

Antibacterial Profile

Sample Efficacy

This screening revealed varying sensitivities of the essential oil at 200 and 100 mg/mL against the tested bacterial strains (Tab. 3).

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Table 3: Sensitivity of bacterial strains to the essential oil, control and antibiotic

	Diameter of inhibition zones			
	EO (mg/mL)		Control	Antibiotic
	200	100	Négative	(Activity)
*S.aureus*ATCC 25923	24±1	23±1	06±0	FOX(30µg) : 23±0 (S)
*S.aureus*2247C/24	22±1	21±1	06±0	FOX (30µg) : 13±0 (R)
*S.sp*2101PIS/24	14±0,5	13±1	06±0	FOX (30µg) : 15±0 (R)
E.coli 2811C/24	06±1	06±1	06±0	FEP(30µg) : 27±0 (S)
K.pneu. 2701C/24	11±1	10±0,5	06±0	FEP(30µg) : 11±0 (R)
*Yer.sp*2340PIS/24	12±1	11±1	06±0	FEP (30µg) : 25±0 (I)
*Sal.*sp 2077AD/24	18±1	16±0,58	06±0	NOR(10µg) : 18±0 (R)
*Sal.*sp 2520C/24	15±1	14±1	06±0	NOR(10µg) : 25±0 (S)

EO: Essential oil from *Cleistopholis patens* collected in Gagnoa; FOX: Cefoxitin; FEP: Cefepime; NOR: Norfloxacin; S: Susceptible; R: Resistant; I: Intermediate

The negative control used (Tween 80 and sterile distilled water) showed no effect against the tested bacterial strains. Regarding the positive controls, the inhibitory effect varied depending on the bacterial strain. Concerning the activity of the essential oil against the strains, no effect was observed against *Escherichia coli* 2811C/24. For the other bacterial strains, the essential oil was active, with inhibition zone diameters ranging from 10 ± 0.5 mm to 23 ± 1 mm at the lower tested concentration of 100 mg/mL, and from 11 ± 1 mm to 24 ± 1 mm at 200 mg/mL. The highest values (23 ± 1 and 24 ± 1 mm) were obtained against *S. aureus* ATCC 25923, demonstrating a positive dose-response relationship. Regarding the sensitivity of the tested bacterial strains to antibiotics, varied responses were observed. Bacteria such as *Staphylococcus aureus* 2247C/24, *Salmonella* sp. 2077AD/24, and *Yersinia* sp. 2340PIS/24, which initially had a wild-type phenotype, appear to have acquired resistance genes to cefoxitin, cefepime, and norfloxacin.[22]

For the bacterial strains against which the essential oil exhibited inhibitory activity, antibacterial parameters including the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined.

Mode of action of the essential oil

The MIC and MBC values determined for the bacterial strains are summarized in Table 4. The MBC/MIC ratio was used to define the mode of action of the essential oil.

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	Antibacterial parameters of the EO			
Bacterialstrains	**MIC (mg/mL)**	**MBC (mg/mL)**	**MBC/MIC**	**Mode of action**
K.pneu. 2701C/24	12.5	25	2	Bactericidal
*Yer.sp*2340PIS/24	6.25	12.5	2	Bactericidal
S. aureus ATCC 29213	6.25	6.25	1	Bactericidal
*S.aureus*2247C/24	6.25	6.25	1	Bactericidal
*S.sp*2101PIS/24	6.25	6.25	1	Bactericidal
*Sal.*sp 2077AD/24	6.25	6.25	1	Bactericidal
*Sal.*sp 2520C/24	12.5	25	2	Bactericidal

Table 4.Antibacterial parameters and mode of action of the essential oil

EO: Essential oil from *Cleistopholis patens* collected in Gagnoa; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

Based on the results presented in the table, the essential oil was bactericidal against the tested strains, with MIC and MBC values ranging from 6.25 to 25 mg/mL. This bactericidal effect may be attributed to the secondary metabolites present in the essential oil, such as terpenic compounds, which are known for their antimicrobial activity.

Conclusion

The study of the essential oil from the leaves of *Cleistopholis patens* collected in Gagnoa allowed the identification of sixteen compounds, representing 95.75% of the total composition. The chemical profile shows a balance between monoterpenes and sesquiterpenes, with a notable proportion of oxygenated sesquiterpenes and the presence of naphthalene. This chemical diversity provides the oil with a broad spectrum of biological potential, including antimicrobial, anti-inflammatory, and antioxidant activities. The combination of these constituents suggests that its efficacy relies on a synergistic effect rather than a single major compound, supporting its potential applications in pharmaceutical, cosmetic, and food industries.

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Conflicts of Interest:

The authors declare no conflicts of interest

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